Volume XIX March, 1925 Number 3

SOIL SCIENCE

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Contents

PUBLISHED MONTHLY
THE WILLIAMS & WILKINS COMPANY
MT. ROYAL AND GUILFORD AVENUES
BALTIMORE, MARYLAND, U. S. A.

Made in United States of America

SOIL SCIENCE

FOUNDED BY
RUTGERS COLLEGE
NEW BRUNSWICK, N. J.

EDITORIAL BOARD

Entered as second-class matter May 12, 1919, at the post office at Baltimore, Maryland, under the act of March 3, 1879

A QUANTITATIVE AND QUALITATIVE DETERMINATION OF THE BACTERIAL FLORA OF SOME REPRESENTATIVE VIRGIN AND CULTIVATED TEXAS SOILS

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Received for publication December 3, 1924

The soils of Texas have never been the subject of any extensive investigation from either a physical, a chemical or a biological standpoint. So far as is known only one investigation of the flora of Texas soil has ever been made. This was by Werkenthin (11), who, in 1916, isolated and identified the predominating moulds in soils of the Austin region. The present paper presents the results of a quantitative and qualitative determination of the bacterial content of some representative virgin and cultivated Texas soils.

Soil bacteriological methods have not yet reached the stage of perfection attained in methods for the bacteriological examination of water, dairy products, foods, and related substances, hence a study of the microorganisms of the soil must of necessity be somewhat limited in its scope. The great variety of organisms and the specialized media required for the growth of certain groups of these, preclude any possibility of ever formulating any one medium which will permit of the growth of all species. The aim of this investigation was to secure the maximum number of forms which would develop under aerobic conditions on a medium designed especially for soil bacteria, no effort being made to isolate either forms with specialized food requirements or anaerobic organisms. A detailed physiological study of all the types isolated was not made.

Very little work seems to have been done in comparing the flora of virgin and cultivated soils. Greaves (6) compared the bacterial activities of 31 samples of Utah soil, 10 of which were virgin, and found with 3 exceptions that a higher total count was obtained from cultivated soil than from virgin. His average count for cultivated soil is almost twice as great as for virgin soil but these figures include moulds as well as bacteria. Qualitative work was not included in this report.

Conn (3), in 1913, compared the bacterial content of 2 soil plats from a qualitative standpoint but both of the plats investigated were under cultivation. The writer has not been able to find any extensive reports of a qualitative bacterial comparison of soils. It is sufficient to mention, in addition to the work of Conn, the contributions of Chester (2), Meyer and his pupils [Neide (8) and others], of Ford and associates (5), and of Barthel (1).

EXPERIMENTAL

Soils investigated. Eight samples of soil were investigated, 4 from the Austin region, and 4 from near Kosse, Limestone County. Samples 1 and 2, representing a soil classified as "Austin clay," were collected in November, 1922. This type of soil is derived chiefly from the weathering of a soft limestone known as "Austin chalk," which belongs to the Cretaceous system. Samples 3 and 4 collected in January, 1923 represent a soil classified as "Yazoo sandy loam," and are river alluvium, recent in age. Because no soil survey has been made in Limestone County as yet, a definite classification of the soils collected there cannot be given. All of the samples from this county were collected in March, 1923. Samples 5 and 6 were from a soil which resembles very much the Houston clay in the Austin region and which is derived from the same geological formation (midway formation, lower Eocene system). Samples 7 and 8 are probably Susquehanna fine sandy loam, a product of the disintegration of the Wilcox formation, also of the Eocene system. They were collected only a few miles from an area which has been mapped as Susquehanna fine sandy loam and, inasmuch as there is no apparent difference in the soils of the two areas, it is believed that the opinion stated above is correct.

Sampling soil. Samples were collected by means of a sampling tube, which was dipped in alcohol and air dried before collecting the sample. The depth of sampling was about 4 inches. The soil was placed immediately into a sterile, corked bottle for transfer to the laboratory. Conn (3), in 1913, considered it unnecessary to use absolutely sterile tools in sampling soil, and Lipman and Martin (7) have since shown that there is no significant dissimilarity between counts of soils taken by aseptic means and those collected without observing the usual precautions to prevent contamination. It was thought best, however, to avoid insofar as possible, the introduction of extraneous organisms, hence all practicable aseptic precautions were observed.

Diluting. As soon as possible after being brought to the laboratory, the soil was emptied into a sterile dish and thoroughly mixed by means of a flamed spatula. One gm. of the soil was then weighed on a chemical balance and mixed with 100 cc. of sterile water in an Erlenmeyer flask. The flask was vigorously shaken for about five minutes in order to bring as many of the bacteria into suspension as possible. After giving the heavier soil particles time to settle out, 1 cc. was removed from this primary dilution and added to 99 cc. of sterile water, giving a dilution of 1:10,000. Further dilutions of 1:100,000 and 1:200,000 were prepared from this 1:10,000 dilution. Aseptic precautions were observed throughout in the preparation of these dilutions. The soil was never left exposed to the air for more than a few seconds at a time, nor were the flasks left open any longer than was necessary. Samples were also weighed and dried to constant weight in an electric oven at 110°C., in order that computations might be made on a dry basis.

Plating. One cc. of the proper dilution (1:100,000 and 1:200,000 being

the dilutions used) was placed in a sterile Petri dish, and the melted agar or gelatin, cooled to 42°C., was added, the contents of the dish being thoroughly mixed by tilting the plate. Five plates of each dilution were made.

Media used. Several media were tried on the first samples plated in order to ascertain which would give the maximum count and still not permit the growth of too many moulds. Soil extract agar, Conn's sodium asparaginate agar (4), and the modified Brown's albumen agar given by Waksman and Fred (10) were compared. Gelatin plates, using two kinds of gelatin were compared, one the tap-water formula of Conn (4), the other a soil-extract gelatin. This medium, however, proved so inferior to agar from a quantitative standpoint that its qualitative value was disregarded, and no further use of gelatin as a plating medium was made. The maximum count was obtained from the albumen agar. This medium is not perfect, in that moulds may develop to such an extent as to interfere with the bacterial count, but it was less

TABLE 1

Bacterial plate count after incubation

SAMPLE	SOURCE	WATER	BACTERIA PER GRAM OF DRY SOIL
		per cent	
1	Virgin Austin clay	23.10	15,020,000
2	Cultivated Austin clay	12.04	5,280,000
3	Virgin Yazoo sandy loam	2.88	2,574,000
4	Cultivated Yazoo sandy loam	0.44	1,614,000
5	Virgin Houston clay (?)	23.35	940,000
6	Cultivated Houston clay (?)	19.35	1,280,000
7	Virgin Susquehanna fine sandy loam (?)	3.60	829,000
8	Cultivated Susquehanna fine sandy loam (?)	7.63	560,000

subject to overgrowth by moulds than were the other media used. Never more than three or four plates out of any one lot were overgrown.

Incubation. The plates were incubated at 25°C. for 7 days. No experimental counts were made but it is believed that this is a sufficiently long period of time to permit of the development of all organisms that are going to develop.

Quantitative study. After incubation the plates were counted, use being made of a binocular microscope when necessary to differentiate between *Actinomycetes* and bacterial colonies, and the number of bacteria per gram of dry soil was computed. The plates in the main gave counts which agreed reasonably closely. Computations were made from the average of plates which gave parallel counts, a plate giving a count abnormally higher or lower than the other plates of the series being disregarded. The results are given in table 1.

Qualitative study. After counting, pure cultures were isolated from the plates for qualitative study. No effort was made to determine the percentage of the different types because it was felt that such an undertaking would

be too liable to error to be of any value. More than one type of organism has been observed to give plate colonies quite similar in appearance. Frequently also, organisms of the same type produce colonies sufficiently dissimilar to introduce a doubt as to whether or not the organisms are the same. An effort was made, however, to isolate one or more pure cultures of each type of colony appearing on the plates. One hundred and twenty-four cultures were isolated, the characteristics of the plate colony being noted at the time of isolation. Purity of culture was checked by microscopical examination and mixed cultures were purified by plating out. It was realized at the start that a detailed study of each organism isolated would not be practicable. Therefore, only the more salient characteristics were determined.

Media used. The cultures were carried on neutral or slightly acid extract agar. Sugar-free broth was used in the preparation of fermentation tubes, 1 per cent of the fermentable substance and 1 per cent of Andrade's indicator being added. The ability to reduce nitrates was tested in a modified Giltay and Aberson's solution, the formation of nitrite being tested for by means of the sulfanilic acid-alpha-naphthylamine hydrochloride method. Potato starch digestion was tested by the addition of potato starch to nutrient agar and pouring plates. After 48 hours' incubation the plates were flooded with a solution of iodine, and any unstained area was measured. Gelatin liquefaction was tested in ordinary nutrient extract gelatin.

Incubation. In all cases, with the exception of starch plates already noted, 2 weeks of incubation at 25°C. were allowed. Readings were made at the end of 1 week and again at the end of 2 weeks.

Microscopical examination. Examination for motility was made on an 18-hour broth culture, negatives being checked by a second examination. Smears for staining by Gram's method were made on agar cultures not over 24 hours old. If no spores were noted in these mounts, older cultures were examined for spores, in some cases cultures as old as 30 days being used. Measurements were made by means of an eye-piece micrometer.

Results. The dominant group of organisms possesses many characteristics in common with the cereus group of Ford (5). However, there was some variation within the group, mainly with regard to the fermentation of glycerol and lactose; therefore it is thought not advisable to state definitely that all of the organisms classed in this physiological group are *Bacillus cereus.* The group occurring second in frequency seems to be *Bacillus mesentericus.* In addition to these, other spore-bearing bacilli isolated and identified were *Bacillus mycoides*, *Bacillus vulgatus* and *Bacillus terminalis.* Seven cultures similar to *Bacillus fusus* and 5 similar to *Bacillus prausnitzii* were isolated, but for each of these groups there is some variation from the type description, either in gelatin liquefaction, starch digestion, nitrate reduction, or lactose fermentation. Twelve cultures of spore-forming bacilli, falling into 4 physiological groups, were isolated but not identified. Qualitative study of the non-spore forming, rod-shaped organisms was less satisfactory than was the

study of the spore bearers, due to non-reactivity in most of the media used. Growth was secured in all media, but the forms are, in the main, so inert as to render classification extremely difficult. The form most frequently isolated was similar to *Serratia rosea*, with the single exception that it did not reduce nitrates. Altogether 40 cultures of non-spore forming, rod-shaped organisms were isolated, these being grouped into 18 physiological groups. Only 6 cultures of cocci were isolated, 2 of which were classified as *Sarcina subflava*. The other 4 were not classified as to species but were separated into 2 physiological groups.

DISCUSSION AND SUMMARY OF RESULTS

On the basis of the results obtained it does not seem likely that, under the conditions of this investigation, there is any qualitative difference between the flora of virgin soil and of cultivated soil. Types frequently encountered have been found to occur in both. Of the types less frequently encountered, too few representatives were isolated to warrant any conclusion.

With one exception the virgin soil showed more bacteria per gram of soil than did the cultivated soil. This fact may possibly be due to the accumulation of toxic products from long-continued cultivation to the same crop. Crop rotation is not extensively practised in Texas and, so far as is known, the crop has not been varied for a number of years on any of the soils investigated. The cultivated soil which gave the higher count has been in cultivation only a few years, whereas the other soils have been cultivated for many years.

The failure to isolate *Bacillus subtilis* is noteworthy. This organism is presumably relatively common in the soil, but in no case was it isolated. It has never been observed to appear as a contamination on plates in the laboratory, whereas the other common spore formers are frequently recovered from air exposure plates. It seems likely that *Bacillus subtilis* is less common in this section than in other sections were qualitative examinations of soil have been made.

Strictly aerobic bacteria seem to be less common in soil than the facultative forms, only 29 cultures altogether having been isolated. This condition is not surprising in view of the statement by Rahn (9) that "strictly aerobic bacteria will never find optimum conditions of existence in soils."

No gas producing forms were isolated. This was contrary to what was expected since gas formers have been isolated from soil by a number of investigators.

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THE FERTILIZER NUTRIENTS REQUIRED BY BARLEY, WHEAT AND OATS, AS SHOWN BY BOTH SOIL AND WATER CULTURES[1]

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Received for publication December 22, 1924

INTRODUCTION

While it is known that plants will grow under widely varying conditions, the amount of certain mineral nutrients which are necessary for growth under such varying conditions as those which exist in soil and in artificial nutrient solutions is not a matter of common knowledge, even among specialists in plant nutrition.

A correlation of plant growth in soil, sand and solution has been made in only a few instances, in spite of the fact that these three growth media have been extensively used for the past 40 years. Shive (18) compared the growth of certain plants by means of both sand and water cultures. McCall (12) made a similar comparison and secured results opposite to those obtained by the former, although each used the same type of nutrient solution. Loew and May (11) tested the validity of the former's theory concerning the lime-magnesia ratio, and incidentally compared the growth of several crops by soil, sand and water cultures. The recent research of Hoagland and Martin (10), on the growth of barley in soil, sand and solution, is perhaps the most complete study to date, because of the fact that the three media were compared, and particular attention was paid to the absorption of the nutrients, the relative growth, and the yield of grain by the plants, in each of the three.

The experiment reported in the present paper was essentially a comparison of soil and water cultures of certain cereals. This comparison was made chiefly on the basis of the nutrient requirements of these cereals for nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O) in both culture media. Certain factors, such as total growth as measured by the weight of air-dry matter produced, the yield of grain, and certain general aspects of growth such as the height of the plants and the color of the foliage were considered in making the correlation.

The relationship of certain elements to nitrogen, phosphoric acid and potash was investigated to some extent, and the problem of the possible toxicity of certain metals was touched upon.

The word "normality" as used in discussions of plant growth, implies a ref-

[1] Contribution 313 of the Rhode Island Agricultural Experiment Station, Kingston.

erence to some standard, and as none seems possible of adoption, the phrases "optimum growth" and "sub-optimum growth" have been used in this article. The former term does not necessarily mean the largest production of dry matter per unit of absorbed nutrients. The yield of grain per unit weight of straw, which is a factor of economic importance, has been considered, as well as the increase in production of dry matter with increased applications of either N, P_2O_5 or K_2O. The term "optimum growth" with respect to any one of the three nutrients mentioned may be defined as that production of dry matter which was not materially increased by additional increments of that nutrient and in which the latter had no effect upon the ratio of straw to grain. The phrase "sub-optimum growth" is self-explanatory and its use here will be understood from what has been previously said.

Under the heading "Limiting condition assumed," in tables 1 to 3, the words "Optimum N, P_2O_5, K_2O," or "Sub-optimum N," etc., imply that the amounts of these nutrients added were estimated to be sufficient for "optimum growth," or deficient enough in a single nutrient to result in "sub-optimum growth."

PLAN OF THE EXPERIMENT

During the season of 1922, barley, wheat and oats were grown to maturity in 8 by 8 inch Wagner pots placed on the bench in the greenhouse. Cereals of the same species and from the same lot of seeds were grown to maturity simultaneously in jars of nutrient solution placed beside the Wagner pots. In each pot and jar 10 selected plants were grown.

The soil in the pots was kept at optimum moisture content in so far as it was possible to gauge this condition from previous work with similar cultures.

Such environmental factors as temperature of the air, humidity, and amount of sunlight, were the same for both series of cultures.

It was not planned to balance carefully the amounts of the fertilizer nutrients supplied to the cereals against the amounts of the same which were removed by the plants. The applications of nitrogen, phosphoric acid and potash made to the pot and jar cultures of each cereal were intended to produce "optimum" and slightly "sub-optimum" growth conditions for these nutrients. It was only necessary that the application of these nutrients be sufficient or insufficient for "optimum growth" of the plants in each medium. With this end in view, no allowance was made for the percentages of nitrogen, phosphoric acid or potash in the seedlings selected for the water cultures, or the amounts of these in the roots of the plants from any of the cultures. The roots were therefore discarded, and only the tops or "above-ground portions" of the plants were considered in making such comparisons of yields and analytical determinations as are recorded in the tables.

It was known that the soil used contained each of these nutrients to some extent, but the amount of these which would be available could not be closely estimated, and for that reason a well-defined deficiency of potash was not attained with any of the pot cultures.

At the conclusion of the growth period the straw and grain from the soil and water cultures of each cereal were weighed. From these weights the cultures which represented "optimum" and "sub-optimum" growth conditions with respect to each nutrient were selected and analyzed for that nutrient. On the basis of these percentages the comparative fertilizer nutrient requirements of each cereal in each of the growth media were estimated.

The different planes of nutrition were established among the cereals grown in the soil by varying the amounts of nitrate of soda, acid phosphate and sulfate of potash applied to the soil medium. With the water cultures, the "optimum" and "sub-optimum" conditions for each nutrient were made possible by regulating the additions of the stock solutions of calcium nitrate, monocalcium phosphate and potassium chloride made to the basal nutrient solution of the other essential ingredients.

Swanhals barley, Marquis spring wheat, and Wasu oats were the cereal varieties which were selected for the experiment.

The "suckers" or "tillers" put out by the plants were cut back from time to time in order to keep the plants to a single stem. These "tillers" were saved and added to the plants when they were harvested. Variations between duplicate cultures in character and amount of growth were thus greatly reduced. Viewed from another angle, the average results from a few pots or jars so treated are as significant as the average results from a large number of soil or water cultures of cereals which are allowed to "tiller" freely.

It is true that a plant so treated is not an average plant, but it should be recognized that plants which are allowed to "tiller" freely are also not comparable with plants which are grown close together in the field, since "tillering" is suppressed under these conditions. Since the cereals of both the soil and water cultures were dealt with similarly, the two methods of culture are comparable.

THE SOIL CULTURES

The growth medium which was used in the Wagner pots consisted of soil and sand. The sand was used in an attempt to produce the desired deficiencies of potash and phosphoric acid. Seven pounds of screened soil from a no-potash plot of the experiment station farm and 11 pounds of sand were thoroughly mixed and put into each Wagner pot.

All of the acid phosphate and one-third or one-half of the nitrate of soda and sulfate of potash were added in solid form to the soil in the pots and thoroughly mixed with it before the seeds were planted. The remaining increments of the two latter chemicals were added in solution. The total amounts of these fertilizer chemicals which were added to each pot are given in column 3 of tables 1 to 3.

The seeds were sown in the pots of soil on March 9. Later the seedlings were thinned to 10 plants per pot. On April 6 the second applications of 1 gm.

TABLE 1

Comparison of soil and solution cultures of barley on the basis of the nutrients added per culture; average air-dry weight of straw and grain produced per culture of 10 plants, and ratio of straw to grain

CULTURE NUMBER		FERTILIZER INGREDIENTS ADDED TO EACH CULTURE		HEIGHT OF PLANTS OF EACH CULTURE		AIR-DRY WEIGHT OF STRAW AND GRAIN PER CULTURE OF 10 PLANTS		AIR-DRY WEIGHT OF STRAW PER CULTURE OF 10 PLANTS		AIR-DRY WEIGHT OF GRAIN PER CULTURE OF 10 PLANTS		RATIO OF STRAW TO GRAIN PER PLANT	
Soil	Solution	Soil	Solution	Soil	Solution	Soil	Solution	Soil	Solution	Soil	Solution	Soil	Solution
		gm.	*gm.*	*feet*	*feet*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*		
44	11	3 nitrate of soda	0.656 N	3.5	3.5	50.2	46.3	32.0	31.7	18.2	14.6	1.8	2.2
45	12	10 acid phosphate	0.140 P_2O_5	3.5	3.5	53.3	41.5	34.2	31.0	19.1	10.5	1.8	3.0
		3 sulfate of potash	0.580 K_2O										
50		2 nitrate of soda		3.2		37.7		24.7		13.0		1.9	
51		10 acid phosphate		3.2		38.8		25.2		13.6		1.9	
		3 sulfate of potash											
48	13	1 nitrate of soda	0.250 N	2.7	2.5	25.7	24.9	15.7	17.1	10.0	7.8	1.6	2.2
49	14	10 acid phosphate	0.140 P_2O_5	2.7	2.5	26.1	25.9	17.9	16.4	8.2	9.5	2.2	1.7
		3 sulfate of potash	0.580 K_2O										
46		———		2.1		12.9		7.8		5.1		1.5	
47		10 acid phosphate		2.1		13.6		8.5		5.1		1.7	
		3 sulfate of potash											
56	9	3 nitrate of soda	0.656 N	3.2	3.5	43.3	44.2	27.5	30.9	15.8	13.3	1.7	2.3
57	10	5 acid phosphate	0.095 P_2O_5	3.2	3.5	45.5	43.7	28.4	30.1	17.1	13.6	1.7	2.2
		3 sulfate of potash	0.580 K_2O										
54	7	3 nitrate of soda	0.656 N	3.2	3.5	43.8	34.7	28.4	23.4	15.4	11.3	1.8	2.1
55	8	2.5 acid phosphate	0.050 P_2O_5	3.2	3.5	45.2	33.3	28.2	22.2	17.0	11.1	1.7	2.0
		3 sulfate of potash	0.580 K_2O*										

52	5	3 nitrate of soda	0.656 N	3.2	3.5	42.7	31.3	27.5	22.6	15.2	8.7	1.8	2.6
53	6	3 sulfate of potash	0.580 K_2O	3.2	3.5	43.0	38.4	26.7	26.3	16.3	12.1	1.6	2.2
42		3 nitrate of soda 10 acid phosphate		3.5		46.2		28.7		17.5		1.6	
43		1 sulfate of potash		3.5		48.3		30.5		17.8		1.7	
40	15	3 nitrate of soda	0.656 N	3.5	3.5	45.2	33.7	29.6	24.7	15.6	9.0	1.9	2.7
41	16	10 acid phosphate	0.140 P_2O_5 0.160 K_2O	3.5	3.5	42.3	35.5	26.8	24.6	15.5	10.9	1.7	2.3
	1		0.250 N		2.5		21.4		14.6		6.8		2.1
	2		0.050 P_2O_5 0.160 K_2O		2.5		23.6		15.0		8.6		1.7
	3		0.250 N		2.5		22.5		16.5		6.0		2.8
	4		0.050 P_2O_5†		2.5		22.8		16.9		5.9		2.9
Average ratio												1.8	2.3

* Potash applied mostly in potassium silicate.

† 1000 p.p.m. extra salt added, as sodium chloride plus calcium sulfate.

TABLE 2

Comparison of soil and solution cultures of wheat on the basis of the nutrients added per culture; average air-dry weight of straw and grain produced per culture of 10 plants, and ratio of straw to grain

CULTURE NUMBER		FERTILIZER INGREDIENTS ADDED TO EACH CULTURE		HEIGHT OF PLANTS OF EACH CULTURE		AIR-DRY WEIGHT OF STRAW AND GRAIN PER CULTURE OF 10 PLANTS		AIR-DRY WEIGHT OF STRAW PER CULTURE OF 10 PLANTS		AIR-DRY WEIGHT OF GRAIN PER CULTURE OF 10 PLANTS		RATIO OF STRAW TO GRAIN PER PLANT	
Soil	Solution	Soil	Solution	Soil	Solution	Soil	Solution	Soil	Solution	Soil	Solution	Soil	Solution
		gm.	*gm.*	*feet*	*feet*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*		
62	43	3 nitrate of soda	0.656 N	3.5	4.5	42.7	48.5	28.6	34.0	14.1	14.5	2.0	2.3
63	44	10 acid phosphate	0.140 P_2O_5	3.5	4.5	42.9	50.2	30.0	36.5	12.9	13.7	2.3	2.7
		3 sulfate of potash	0.580 K_2O										
68		2 nitrate of soda		3.5		36.3		25.8		10.5		2.5	
69		10 acid phosphate		3.5		37.7		23.5		14.2		1.7	
		3 sulfate of potash											
66	45	1 nitrate of soda	0.250 N	3.5	3.7	26.9	26.8	17.9	18.9	9.0	7.9	2.0	2.4
67	46	10 acid phosphate	0.140 P_2O_5	3.5	3.7	27.6	30.7	18.2	20.9	9.4	9.8	1.9	2.1
		3 sulfate of potash	0.580 K_2O										
64		———		2.5		12.0		8.1		3.9		2.1	
65		10 acid phosphate		2.5		13.7		9.3		4.4		2.1	
		3 sulfate of potash											
74	41	3 nitrate of soda	0.656 N	3.5	3.7	40.6	42.5	25.1	32.6	15.5	9.9	1.6	3.3
75	42	5 acid phosphate	0.095 P_2O_5	3.5	3.7	40.7	43.0	26.5	32.3	14.2	10.7	1.9	3.0
		3 sulfate of potash	0.580 K_2O										
72	39	3 nitrate of soda	0.656 N	3.5	3.7	39.2	34.2	25.2	26.0	14.0	8.2	1.8	3.2
73	40	2.5 acid phosphate	0.050 P_2O_5	3.5	3.7	39.1	32.1	24.0	24.7	15.1	7.4	1.6	3.3
		3 sulfate of potash	0.580 K_2O*										

70	37	3 nitrate of soda	0.656 N	3.5	3.7	35.1	32.5	21.5	24.4	13.6	8.1	1.6	3.0
71	38	3 sulfate of potash	0.050 P_2O_5 0.580 K_2O	3.5	3.7	33.2	33.6	20.6	26.0	12.6	7.6	1.6	3.4
60		3 nitrate of soda		3.5		41.7		27.1		14.6		1.9	
61		10 acid phosphate 1 sulfate of potash		3.5		45.7		28.3		17.4		1.6	
58	47	3 nitrate of soda	0.656 N	3.5	3.7	41.6	35.1	26.5	26.2	15.1	8.9	1.8	2.9
59	48	10 acid phosphate	0.140 P_2O_5 0.160 K_2O	3.5	3.7	42.9	37.6	26.8	26.9	16.1	10.7	1.7	2.5
	33		0.250 N		3.7		25.9		18.8		7.1		2.6
	34		0.050 P_2O_5 0.160 K_2O		3.7		24.0		17.0		7.0		2.4
	35		0.250 N		3.7		23.9		17.6		6.3		2.8
	36		0.050 P_2O_5† 0.160 K_2O		3.7		23.9		18.3		5.6		3.3
Average ratio												2.1	2.5

* Potash applied mostly in potassium silicate.

† 1000 p.p.m.extra salt added, as sodium chloride plus calcium sulfate.

TABLE 3

Comparison of soil and solution cultures of oats on the basis of the nutrients added per culture; average air-dry weight of straw and grain produced per culture of 10 plants, and ratio of straw to grain

CULTURE NUMBER		FERTILIZER INGREDIENTS ADDED TO EACH CULTURE		HEIGHT OF PLANTS OF EACH CULTURE		AIR-DRY WEIGHT OF STRAW AND GRAIN PER CULTURE OF 10 PLANTS		AIR-DRY WEIGHT OF STRAW PER CULTURE OF 10 PLANTS		AIR-DRY WEIGHT OF GRAIN PER CULTURE OF 10 PLANTS		RATIO OF STRAW TO GRAIN PER PLANT	
Soil	Solution	Soil	Solution	Soil	Solution	Soil	Solution	Soil	Solution	Soil	Solution	Soil	Solution
		gm.	*gm.*	*feet*	*feet*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*		
80	27	3 nitrate of soda	0.656 N	4.0	4.5	57.1	56.2	36.0	37.7	21.1	18.5	1.7	2.0
81	28	10 acid phosphate	0.140 P_2O_5	4.0	4.5	51.0	60.0	36.0	38.9	15.0	21.1	2.4	1.8
		3 sulfate of potash	0.580 K_2O										
86		2 nitrate of soda		4.0		50.7		31.4		19.3		1.6	
87		10 acid phosphate		4.0		52.6		33.4		19.2		1.7	
		3 sulfate of potash											
84	29	1 nitrate of soda	0.250 N	3.5	3.2	38.9	31.3	25.4	20.7	13.5	10.6	1.9	2.0
85	30	10 acid phosphate	0.140 P_2O_5	3.5	3.2	34.9	35.1	23.2	23.8	11.7	11.3	2.0	2.1
		3 sulfate of potash	0.580 K_2O										
82		———		2.1		12.1		8.9		3.2		2.8	
83		10 acid phosphate		2.1		10.7		8.0		2.7		3.0	
		3 sulfate of potash											
92	25	3 nitrate of soda	0.656 N	4.0	4.5	51.5	51.9	31.5	35.9	20.0	16.0	1.6	2.2
93	26	5 acid phosphate	0.095 P_2O_5	4.0	4.5	52.7	50.0	32.6	33.8	20.1	16.2	1.6	2.1
		3 sulfate of potash	0.580 K_2O										
90	23	3 nitrate of soda	0.656 N	4.0	3.7	58.1	37.4	35.5	28.6	22.6	8.8	1.6	3.3
91	24	2.5 acid phosphate	0.050 P_2O_5	4.0	3.7	50.7	37.9	30.6	28.6	20.1	9.3	1.5	3.1
		3 sulfate of potash	0.580 K_2O*										

88	21	3 nitrate of soda	0.656 N	4.0	3.7	51.7	35.8	32.1	27.6	19.6	8.2	1.6	3.4
89	22	3 sulfate of potash	0.050 P_2O_5 0.580 K_2O	4.0	3.7	51.2	35.4	32.4	27.0	18.8	8.4	1.7	3.2
78		3 nitrate of soda		4.0		54.5		34.5		20.0		1.7	
79		10 acid phosphate 1 sulfate of potash		4.0		58.8		36.0		22.8		1.6	
76	31	3 nitrate of soda	0.656 N	4.0	3.7	54.8	43.6	34.6	33.1	20.2	10.5	1.7	3.2
77	32	10 acid phosphate	0.140 P_2O_5 0.160 K_2O	4.0	3.7	53.2	46.4	33.3	31.0	19.9	15.4	1.7	2.0
	17		0.250 N				30.3		21.2		9.1		2.3
	18		0.050 P_2O_5 0.160 K_2O				31.0		22.0		9.0		2.4
	19		0.250 N†				30.2		19.3		10.9		1.8
	20		0.050 P_2O_5 0.160 K_2O				29.6		20.0		9.6		2.1
Average ratio												1.9	2.4

* Potash applied mostly in potassium silicate.

† 1000 p.p.m. extra salt added, as sodium chloride plus calcium sulfate.

each of nitrate of soda and sulfate of potash were made to the pots designated to receive 2 or 3 gm. of these chemicals. The third and final increments of these salts were applied on April 21 to the pots which were to receive a 3-gm. application.

The growth period of the barley was 14 weeks and that of the wheat and oats approximately 15 weeks, since the former cereal was harvested on June 14 and the latter two were allowed to grow until June 21 and June 24 respectively.

The pH of the soil medium as it was prepared was not determined. It seems probable, however, on the basis of previous determinations made on soil samples[2] from the plot from which this was taken, that the H-ion concentration was not greater than that indicated by a pH of 6.0. Hoagland (9) found that a degree of acidity represented by a pH value of 5.0 was not inhibitive to the growth of barley seedlings in soil.

THE WATER CULTURES

The methods employed by Pember (15) in experiments to determine the nutrient requirements of barley were closely followed in conducting the water cultures of this experiment.

The stock solutions were made from the following so-called C. P. chemicals: mono-calcium phosphate, potassium chloride, calcium nitrate, potassium silicate, magnesium sulfate, ferric nitrate, sodium chloride, and calcium sulfate. Well water was used as the solvent in making up the nutrient solutions.

Biological specimen jars of 3300-cc. capacity, painted on the outside with black asphaltum paint, and over this with aluminum paint, were used as culture vessels. Three liters of nutrient solution were used in each jar, and the solutions were changed each week for the first 11 weeks that the plants were in the jars. At the end of the eleventh week the nutrient solutions were replaced by well water.

The amounts of the magnesium sulfate and ferric nitrate which were added to each jar at each change of the solution were constant. The stock solution of the former salt contained 24.6 mgm. per cc. and 25 cc. were added each week. The ferric nitrate was applied as follows: 2.86 gm of this salt were dissolved in 200 cc. of water. Ten cc. of this solution was diluted to 500 cc. and 10 cc. of the latter added to each of the water cultures every week.

Certain brief statements concerning the total concentration of the nutrient used here will perhaps afford a basis of comparison between the water cultures of this experiment and the water cultures which have been used in other plant-nutrition studies.

This concentration is stated only within approximate limits, since it was dependent not only upon the amount and rate of application of the salts which furnished the fertilizer nutrients, but also upon the concentration of the well

[2] Determinations made by Dr. Paul S. Burgess, formerly Chemist, R. I. Agricultural Experiment Station.

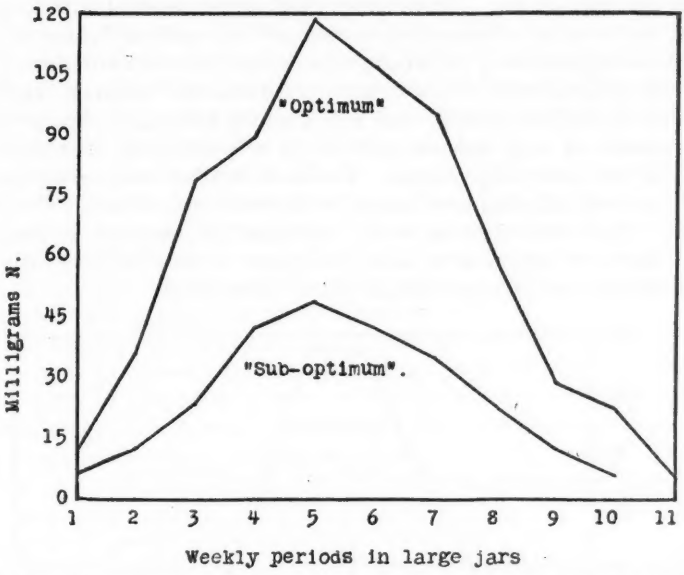

FIG. 1. THE AMOUNTS OF N APPLIED TO THE WATER CULTURES OF BARLEY, WHEAT AND OATS

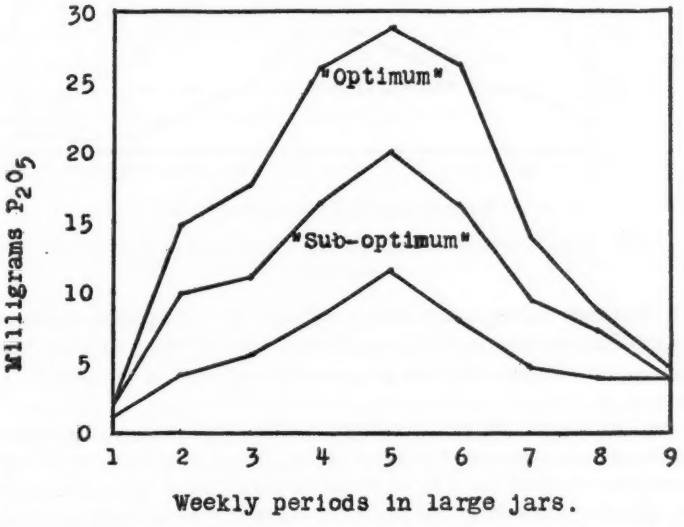

FIG. 2. THE AMOUNTS OF P_2O_5 APPLIED TO THE WATER CULTURES OF BARLEY, WHEAT AND OATS

water used as a solvent. The minimum initial concentration of nutrient salts added to the water cultures which represented "sub-optimum" planes of nutrition was approximately 175 p.p.m., while the maximum concentration of the nutrient solutions added to the cultures which received "optimum" applications of the fertilizer nutrients was approximately 850 p.p.m. The average concentration of total dissolved solids in the well water was determined by analysis, and found to be 95 p.p.m. The outside limits of total concentration of the nutrient solutions were, except in one group of 6 cultures, 270 to 945 p.p.m. The 6 water cultures which constituted the exception to the previous statement were those to which 1000 p.p.m. of extra salt (782 parts sodium chloride and 218 parts calcium sulfate) were added.

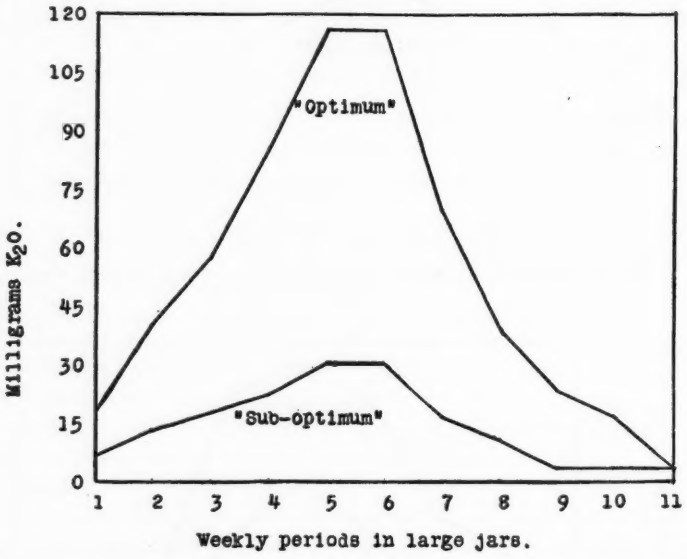

FIG. 3. THE AMOUNTS OF K_2O APPLIED TO THE WATER CULTURES OF BARLEY, WHEAT AND OATS

The fact that this extra salt had no influence upon the growth of the water cultures of the cereals to which it was added makes it appear that the concentration of the nutrient solutions *per se* was not a factor in the growth of the plants.

From the second change of nutrient solution until the conclusion of the experiment, water cultures 7 and 8 of barley, 23 and 24 of oats, and 39 and 40 of wheat received all but 1 cc. of the weekly application of potash in potassium silicate. Conversely, all the other cultures received only 1 cc. of the potassium silicate solution each week.

The weekly applications of nitrogen, phosphoric acid and potash are given

graphically in figures 1 to 3. Since it was known that the well water contained appreciable amounts of potash, the numbers of figure 3 can be taken as representing only the amounts of potash supplied to the plants in addition to the amounts supplied by the well water. The applications of nitrogen and phosphoric acid as given in figures 1 and 2 respectively may be considered as practically synonymous with the total amounts.

The applications are recorded in the figures as milligrams per culture vessel. By dividing the number expressing these applications by 3 (3 liters of solution per jar) the concentration of the fertilizer nutrients given each week may be obtained as milligrams per liter, which is equivalent to parts per million.

The total amounts of the three fertilizer nutrients applied during the growth of the cereals are given in column 4 of tables 1 to 3.

The seeds for the series of water cultures were germinated on a perforated aluminum disk floated on water by means of attached corks. By March 6 the barley and oat seedlings were sufficiently developed to be transferred to small bottles of a nutrient solution which had a total concentration of dissolved solids equivalent to about 550 p.p.m. The wheat seedlings were similarly transferred the following day, and all three species were "nursed" for 2 weeks in these bottles. At the end of this time 10 plants of uniform size were selected for growth in each of the large jars.

The barley was harvested on June 14, the wheat on June 21 and the oats on June 24. The growth period of the former species was approximately 14 weeks and that of the latter two about 15 weeks, as was the case with the soil cultures.

The reaction of the nutrient solution was not determined prior to its use in the jars nor during its period of contact with the roots of the cereals. From the results of the previous experiments at this station with nutrient solutions of the type here used, it seems safe to conclude that the reaction of the culture solutions did not become a controlling factor.

THE COMPARATIVE GROWTH OF THE CEREALS OF THE SOIL AND WATER CULTURES

By April 7 it was noticeable that the plants in the soil cultures which had received no nitrate of soda were beginning to lag behind the others. Up to April 11 the plants in the soil appeared, generally, to be making a more rapid growth than those in the water cultures.

All of the water-culture seedlings had their third leaf and were about to put out their fourth by April 4. The general appearance of the plants in the water cultures at this time was not indicative of a deficiency of any nutrient.

The lack of nitrogen evidenced by the size of the plants in the soil cultures which had received no nitrate of soda had become more pronounced by April 21. The leaves were light green in color and the plants were only one-third the size of those in the cultures which had received a 3-gm. application of nitrate of soda. In those cultures which had received the 1-gm. and even the 2-gm. applications of this fertilizer chemical, the size of the plants at this time indicated a deficiency of nitrogen.

Pember (15) found that a deficiency of potash in the barley plant was closely correlated with the appearance of rusty brown spots on the oldest leaves. This spotting became more general with more pronounced deficiencies of potash. He observed also that barley plants which were markedly deficient in phosphoric acid were dark green in color at the fifth week. By the eighth week of growth they had a pronounced purple color in the stalks and this coloration extended gradually to the leaves and heads.

In the present experiment none of the plants grown in the soil had begun to show any of the above symptoms of potash or phosphoric acid deficiency by April 21.

By April 24 the wheat and oat plants in the soil had their seventh leaf, and the eighth leaf had appeared on the barley plants. By the above date the plants in the water cultures had caught up with those in the soil cultures in their stage of development. At this time the water-culture plants were ready to put out their eighth leaf. The water cultures to which small amounts of calcium nitrate were added contained shorter plants than were growing in those jars which had been furnished with larger amounts of this salt. The color of all the plants in the water cultures was normal.

Beards appeared on the barley plants and heads were visible on the wheat and oat plants of both the soil and water cultures on May 8, May 15 and May 23, respectively.

The soil cultures of all three cereals showed a lack of nitrogen at this time in the cases before mentioned. No lack of potash was evident with the plants of any soil culture, and the symptoms of phosphoric acid deficiency were questionable.

The initiatory and closing phases of maturity for each of the cereals occurred at the same time in both growth media.

The heights of the plants from both series of cultures are recorded in tables 1 to 3.

With regard to the comparative rate of growth of the cereals in the soil and in the water cultures, it may be stated that the rate of growth was much more rapid in the soil up to the fifth week. After 5 weeks in the large jars, i.e., by the seventh week of growth, the water-culture plants reached a stage of growth equal to that which had been attained by the soil-culture plants, and thereafter the rate of growth was the same in both media.

The plants from each culture were thoroughly air-dried after harvesting. The grain was then separated from the straw, and each weighed separately.

It is apparent that the ratio of straw to grain is the outstanding difference between the cereals from the soil cultures and those from the water cultures. The average ratio is approximately 33 per cent greater for the barley and oats from the water cultures than it is for the barley and oats which were grown in the soil. This ratio is 55 per cent greater with the wheat which was grown in solution than with the wheat which was grown in soil.

When the grain was separated from the straw, it was noted that the indi-

vidual kernels of grain from many of the cereal plants grown in the water cultures were not so plump as the kernels from the cereal plants grown in the soil. The former kernels had a shriveled appearance in many cases. The characterization of these two types of kernels as "plump" and "shriveled" is probably synonymous with the designations " starchy" and "flinty" which have been used by certain workers to distinguish between two grades of kernels frequently yielded by field-grown wheat.

Harper and Peter (7) observed that certain varieties of wheat had a tendency to produce kernels which were "flinty and angular in outline," while other varieties of this cereal yielded "plump, starchy" kernels. A study of the chemical composition of these two types of kernels brought out the fact that the former always contains more protein. Subsequent work did not confirm the idea that the flinty kernels were typical of a condition of immaturity.

It is to be expected, perhaps, that the grain from the cereals grown in solution would be higher in nitrogen than the grain from plants grown in soil, since in artificial culture solutions a higher concentration of nitrates is probably maintained until near to the time of maturity of the plant. Hoagland and Martin (10) found that the concentration of nitrates in the soil solution from under a growing crop of barley was zero after 8 weeks growth of the plants. Burd (3) under similar conditions noted that the concentration of nitrates dropped from an initial value of 69 p.p.m. to 2 p.p.m. at the eighth week of growth.

Figure 1 shows that in the nutrient solutions used in the water cultures of the present experiment, the concentration of nitrogen in the "sub-optimum" nitrogen cultures was approximately 14 p.p.m. at the eighth week of growth (the sixth week in the large jars), while the concentration of this nutrient in the "optimum" nitrogen cultures was about 35 p.p.m. at the above period. The concentration of nitrogen did not become zero until two or three weeks prior to the time of harvesting the plants.

It is conceivable that such differences in the concentration of nitrogen in the soil solution and in the nutrient solutions generally used in water cultures may have a bearing upon the percentage of protein in the grain, and also, upon the ratio of straw to grain in cereals grown in soil and in solution.

THE CULTURES SELECTED FOR ANALYSIS

On the basis of the data contained in tables 1 to 3 those cultures of barley, wheat and oats which seemed to have made a "sub-optimum" growth, due to a deficiency of one of the three nutrients, were selected and analyzed for the nutrient which was considered to be insufficient.

The cereals selected, therefore, were those from the soil and from the water cultures in which an increase in the applications of nitrogen, phosphoric acid or potash was accompanied by *bona fide* differences in the amount of growth made by the plants. The amount of growth was measured by the weight of dry matter in the tops of the plants.

Moisture determinations were made on all samples. The nutrient requirements are expressed as percentage in dry matter of the plants.

The following example will serve as an illustration of the basis and method used in selecting the plants for analysis. The wheat in cultures 62 and 63 received an application of 3 gm. of nitrate of soda, the wheat in cultures 68 and 69 received 2 gm. of this fertilizer chemical, while only 1 gm. was applied to the wheat in cultures 66 and 67. The applications of acid phosphate and sulfate of potash were the same in all three of the above cases. When the application of nitrate of soda was lowered from 3 to 2 gm. as in cultures 68 and 69, the production of dry matter dropped approximately one-seventh. When only 1 gm. was applied (cultures 66 and 67) the production of dry matter was depressed about one-third. The weights of both straw and grain were appreciably lower in the latter case. The plants in cultures 66 and 67 were undoubtedly limited in growth by a lack of nitrogen. The wheat in cultures 68 and 69 appeared to have taken up slightly less nitrogen than was required for "optimum" growth. Increasing the application by one-half did not markedly increase the weight of dry matter produced by the plants in cultures 62 and 63 over the weight produced in cultures 68 and 69. The wheat which was grown in cultures 62 and 63 was considered to have made an "optimum" growth, and the wheat cultures 68 and 69 were regarded as having made a slightly "sub-optimum" growth, due to a lack of nitrogen.

The plants from these cultures were analyzed for nitrogen, and the percentages thereof in the dry matter of the wheat from cultures 68 and 69 were regarded as the lower limit to the amount of nitrogen required for the "optimum" growth of wheat in the soil.

The cultures selected for analysis, and the conditions which they were chosen to represent, are given in tables 4 to 6.

Cultures 42 and 43 of barley, 60 and 61 of wheat, and 78 and 79 of oats, grown in soil, were analyzed for nitrogen, phosphoric acid and potash, as these were considered to be nearly "optimum" in their growth. These are not strictly comparable to the "sub-optimum" cultures, however, as they were given one-third the application of potash given to the "sub-optimum" phosphoric acid and nitrogen cultures.

ANALYTICAL METHODS USED

The straw and grain from each culture of plants were re-combined after these two components of the air-dry matter had been separately weighed, and the entire above-ground portion of the plant was ground for analysis.

The following analytical methods were used:

Nitrogen. The standard Kjeldahl method for the determination of organic nitrogen, as given in "Methods of Analysis" (2) was used.

Potash and phosphoric acid. The samples were prepared for the determination of potash and phosphoric acid by the wet digestion method used by Ames and Boltz (1). The usual gravimetric method was used to determine the percentage of phosphoric acid. The per-

centage of potash was determined by the Lindo-Gladding (2) method, except that calcium was not precipitated as the oxalate prior to taking the aliquot for the potash determination. The method as thus amended gave results which were like those secured by the standard method of procedure.

Calcium and magnesium. The percentage of calcium oxide was determined volumetrically. In the determination of magnesium oxide the method outlined by Patten (14) was followed.

Silica. The method given in Talbot's Quantitative Analysis (19) for the determination of silica and insoluble matter in limestone was used. The percentage of silica and insoluble matter was taken as equivalent to the percentage of silica, since the amount of insoluble matter, i.e. dust and dirt, adhering to the plants is usually a negligible factor in carefully handled greenhouse cultures.

Iron and aluminum. Considerable difficulty was encountered in finding a satisfactory method for the determination of the very small amounts of the oxides of iron and aluminum which were present in the plants. The method recommended by Patten (14) for the determination of the combined phosphates of these metals was finally so modified that the separate percentages of these phosphates could be determined.

The modified method used was as follows: The ferric and aluminum phosphates were precipitated together and ignited to constant weight, according to the method referred to above. Then 8 cc. of 1:1 sulfuric acid was added to this precipitate, and the crucible containing it was heated on a steam bath from one to two hours. The contents were transferred to a beaker, diluted, and heated on the water bath from four to six hours, or until the iron was completely dissolved. The solution was removed from the water bath, run through a Jones reductor, cooled and then immediately titrated with 0.05 *N* potassium permanganate.

This method, as outlined above, gave very good check results when tried out in analyzing a synthetic solution of ferric and aluminum phosphates.

NITROGEN, PHOSPHORIC ACID AND POTASH NECESSARY FOR "OPTIMUM" GROWTH OF BARLEY, WHEAT AND OATS

The percentages of nitrogen, phosphoric acid and potash, which were contained in the dry matter of the three cereals when grown in both media, are given in tables 4 to 6, together with the average amounts of these mineral nutrients which were removed in the tops of each plant.

Barley. The weights of dry matter (table 4) produced by the barley grown in jars of solution, with 250 mgm. nitrogen and with 656 mgm. nitrogen, are very similar to the amounts of dry matter from the barley plants grown in the pots of soil with 1- and 3-gm. applications of nitrate of soda, respectively. Two gm. of nitrate of soda produced a weight of dry matter about midway between the weights produced with the 1- and 3-gm. applications of this fertilizer chemical. The percentage of nitrogen in the barley grown in solution was somewhat less than that of plants of similar size and grain-yield but grown in soil. The percentages seem to justify the conclusion that barley which contained 0.9 per cent of nitrogen typifies "sub-optimum" growth conditions with respect to this nutrient.

The barley grown in soil from which acid phosphate was omitted produced an average of 17 per cent less dry matter per plant than the barley grown with a 10-gm. application of acid phosphate. The plants with "sub-optimum"

TABLE 4

Comparison of the amounts of nitrogen, phosphoric acid and potash absorbed by barley tops when the plants were grown in soil and in solution

CULTURE NUMBER		WEIGHT OF DRY MATTER PER CULTURE OF 10 PLANTS		LIMITING CONDITIONS ASSUMED	NUTRIENT CONTENT IN DRY MATTER OF THE TOPS GROWN IN EACH CULTURE						AMOUNT REMOVED FROM EACH CULTURE						AMOUNT ADDED TO EACH CULTURE					
					Soil			Solution			Soil			Solution			Soil			Solution		
Soil	Solution	Soil	Solution		N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O
		gm.	*gm.*		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
42	11	43.2	43.2	"Optimum" N, P_2O_5 and K_2O	1.14	0.63	1.55	0.89	0.32	1.46	0.492	0.267	0.669	0.384	0.138	0.630	0.450	1.700	0.500	0.656	0.140	0.580
43	12	44.6	38.4		0.97	0.64	1.57	0.89	0.35	1.52	0.432	0.280	0.700	0.341	0.130	0.583						
48	13	23.8	23.3	"Sub-optimum" N	0.84			0.77			0.200			0.018			0.150	1.700	1.500	0.250	0.140	0.580
49	14	24.3	27.0		0.84			0.70			0.204			0.168								
50		35.4		"Sub-optimum" N	0.91						0.322						0.300	1.700	1.500			
51		35.9			0.94						0.339											
52	5	40.0	29.5	"Sub-optimum" P_2O_5		0.47			0.17			0.192			0.050		0.450	0	1.500	0.656	0.050	0.580
53	6	39.3	35.5			0.49			0.19			0.184			0.063							
40	15	42.2	31.3	"Sub-optimum" K_2O		0.60	1.02		0.38	0.91		0.249	0.430		0.119	0.284	0.450	1.700	0	0.656	0.140	0.160
41	16	39.3	33.1			0.66	1.22		0.38	0.76		0.255	0.479		0.125	0.251						

phosphoric acid contained 0.5 per cent of phosphoric acid. The weight of grain yielded by the "sub-optimum"phosphoric acid plants was less. The amount of growth made by barley in solution, together with the percentages of phosphoric acid in the plants, indicates that the requirement for the nutrient in this medium was considerably lower than the amounts required by the barley grown in the soil medium. When the application of phosphoric acid made to the solution cultures was lowered from 140 to 50 mgm. a growth depression of 23 per cent resulted. While the larger application may have been more than sufficient to maintain "optimum" growth, the smaller amount was undoubtedly insufficient for this purpose. The average percentage of phosphoric acid in the dry matter of the tops of barley grown in solution under "sub-optimum" conditions was 0.18 per cent. Pember and McLean (16) from average results secured over a period of 5 years, estimate the phosphoric acid requirement of barley grown in solution to be 0.2 per cent in dry matter of the entire plant.

Well-defined potash deficiencies were not attained with the barley grown in the pots. The weights of dry matter from the soil and from the water cultures of barley which were representative of "optimum" growth conditions, and the percentages of potash in this dry matter were very similar. The production of dry matter was not much depressed in the soil when sulfate of potash was not added to the soil medium, although the percentage of potash in the barley under these conditions was reduced to about 1.1. When the percentage of potash in the barley grown in solution dropped to an average of 0.8, the growth was depressed 21 per cent below that of plants containing 1.5 per cent. There are no indications that the average percentage of potash with the largest amount of potash supplied represented the most economical use of this nutrient. It can only be stated that a percentage of 0.8 was too low to maintain "optimum" growth, while an amount equivalent to 1.5 per cent probably represented "luxury consumption" by the plant. Pember and McLean (16) estimate the minimum potash requirement for "optimum" growth of barley including roots to be 0.7 per cent. While the amount given here is appreciably larger than this, it should be remembered that it is based on only one series of water cultures, and is not supported by the soil cultures, while their figure is the average result of five series grown in successive years.

Wheat. The data concerning the amounts of nitrogen, phosphoric acid and potash required for "optimum" growth of wheat as contained in table 5, show that the wheat grown in solution with "optimum" nitrogen made a greater total growth than it made in the soil on a similar plane of nutrition. The "sub-optimum" nitrogen applications of both the soil and the water cultures resulted in weights of dry matter which were very similar, although the percentage of nitrogen in the plants of the latter was about 0.1 less. The results indicate that wheat, like barley, required less nitrogen when grown in solution than it did when grown in soil. The increased total growth in the soil when the application of nitrate of soda was increased from 2 to 3 gm., and

TABLE 5

Comparison of the amounts of nitrogen, phosphoric acid and potash absorbed by wheat tops when the plants were grown in soil and in solution

CULTURE NUMBER		WEIGHT OF DRY MATTER PER CULTURE OF 10 PLANTS		LIMITING CONDITION ASSUMED	NUTRIENT CONTENT IN DRY MATTER OF THE TOPS GROWN IN EACH CULTURE						AMOUNT REMOVED FROM EACH CULTURE						AMOUNT ADDED TO EACH CULTURE					
					Soil			Solution			Soil			Solution			Soil			Solution		
Soil	Solution	Soil	Solution		N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O
		gm.	*gm.*		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
60	43	38.5	45.5	"Optimum" N, P_2O_5 and K_2O	1.26	0.62	1.58	1.14	0.33	1.21	0.485	0.238	0.608	0.518	0.150	0.550						
61	44	42.2	47.0		1.12	0.55	1.47	1.03	0.30	1.15	0.472	0.232	0.620	0.484	0.141	0.540	0.450	1.700	0.500	0.656	0.140	0.580
66	45	24.9	25.2	"Sub-optimum" N	0.92			0.76			0.229			0.191								
67	46	25.7	28.5		0.90			0.80			0.231			0.228			0.150	1.700	1.500	0.250	0.140	0.580
68		33.6		"Sub-optimum" N	1.02						0.342											
69		34.8			1.02						0.354						0.300	1.700	1.500			
70	37	32.0	30.3	"Sub-optimum" P_2O_5		0.67			0.16			0.214			0.048							
71	38	30.8	31.2			0.62			0.20			0.191			0.062		0.450	0	1.500	0.656	0.050	0.580
58	47	38.5	33.3	"Sub-optimum" K_2O		0.55	1.10		0.42	0.69		0.211	0.423		0.139	0.229						
59	48	39.4	35.2			0.56	1.15		0.35	0.64		0.220	0.453		0.123	0.225	0.450	1.700	0	0.656	0.140	0.160

the percentage of nitrogen in culture 61, in which the plants were grown with the larger application, indicate that the wheat in the pots made "sub-optimum" growth when the soil contained 1.0 per cent of nitrogen. The nitrogen content in the dry matter of the wheat grown in solution with a similar "sub-optimum" level of nitrogen would probably have been 0.8 to 1.0 per cent.

It is obvious that the phosphoric acid requirement of the wheat from the soil was different from this requirement for the wheat grown in solution. A similar depression of growth was attained in the soil and in the water cultures, although the growth depression in the soil was due to some factor other than a deficiency of phosphoric acid, as shown by an increased percentage of phosphoric acid in the smaller weight of dry matter. The decreased growth of the water-culture wheat with a low phosphoric acid application, and the comparative phosphoric acid content in dry matter indicate that wheat, under the conditions of this experiment, could not make its "optimum" growth with 0.20 per cent of phosphoric acid in its dry matter, and probably did not require more than 0.30 per cent of this nutrient. No percentage can be advanced to indicate the amount of phosphoric acid required for "sub-optimum" growth of wheat in the soil.

The soil-culture wheat, which it was desired to grow under "sub-optimum" potash conditions was in reality grown with an "optimum" and perhaps a "super-optimum" amount of available potash. This is shown by the fact that the growth was not appreciably depressed when sulfate of potash was not applied to the soil, even though the percentage of this nutrient in the dry matter dropped from an average of 1.53 to 1.13. The crop of wheat produced in solution with the larger application of potash contained an average of 1.18 per cent of this nutrient. The smaller or "sub-optimum" application depressed the growth 26 per cent and reduced the potash content to 0.67 per cent.

The indications are that 1.1 per cent of potash in dry matter of wheat is an amount sufficient to mature a vigorous plant, although the minimum amount necessary for this purpose may be somewhat less.

Oats. Table 6 shows that the amounts of nitrogen, phosphoric acid and potash required for "sub-optimum" growth of oats in both the soil and the water cultures were less than the amounts of these mineral nutrients necessary for "sub-optimum" growth of barley and wheat in the same media.

Both in the amount of dry matter produced, and in the percentage of nitrogen therein, the oat plants of the water cultures, which received 250 mgm. of nitrogen, are like those of the oats in the soil cultures, to which were added 150 mgm. of nitrogen. The application of 300 mgm. of nitrogen to the soil increased the production of dry matter to an amount only slightly less than that which resulted from the oats supplied with 450 mgm. of nitrogen. The oats grown in solution with 656 mgm. of nitrogen produced a crop larger than the oats grown in soil with 300 mgm. of nitrogen, and the percentages of this nutrient were somewhat similar. The oats with "sub-optimum" nitrogen contained 0.6 to 0.7 per cent of this element.

TABLE 6

Comparison of the amounts of nitrogen, phosphoric acid and potash absorbed by oat tops when the plants were grown in soil and in solution

CULTURE NUMBER		WEIGHT OF DRY MATTER PER CULTURE OF 10 PLANTS		LIMITING CONDITION ASSUMED	NUTRIENT CONTENT IN DRY MATTER OF THE TOPS GROWN IN EACH CULTURE						AMOUNT REMOVED FROM EACH CULTURE						AMOUNT ADDED TO EACH CULTURE					
					Soil			Solution			Soil			Solution			Soil			Solution		
Soil	Solution	Soil	Solution		N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O
		gm.	*gm.*		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
78	27	51.1	52.6	"Optimum" N, P_2O_5 and K_2O	0.86	0.80	1.60	0.75	0.26	1.15	0.439	0.408	0.817	0.394	0.136	0.604	0.450	1.700	0.500	0.656	0.140	0.580
79	28	55.4	56.7		0.85	0.74	1.54	0.79	0.25	1.03	0.470	0.409	0.853	0.447	0.141	0.584						
84	29	36.6	30.0	"Sub-optimum" N	0.58			0.53			0.212			0.159			0.150	1.700	1.500	0.250	0.140	0.580
85	30	32.8	33.4		0.55			0.59			0.180			0.197								
86		47.9		"Sub-optimum" N	0.68						0.325						0.300	1.700	1.500			
87		49.9			0.67						0.334											
88	21	48.2	33.2	"Sub-optimum" P_2O_5		0.38			0.15			0.183			0.049		0.450	0	1.500	0.656	0.050	0.580
89	22	48.3	33.7			0.44			0.16			0.212			0.053							
76	31	51.2	41.5	"Sub-optimum" K_2O		0.87	1.18		0.29	0.47		0.445	0.604		0.120	0.195	0.450	1.700	0	0.656	0.140	0.160
77	32	49.4	43.4			0.85	1.22		0.27	0.47		0.419	0.602		0.117	0.203						

The amounts of dry matter produced by the oats of the water cultures which represent "optimum" growth conditions were about equal to the amounts of dry matter produced under "optimum" conditions in soil, but the water cultures absorbed only about one-third as much phosphoric acid as was taken up by the soil cultures. The amount of this nutrient removed by the plants of the "optimum" water cultures is probably about the percentage in dry matter necessary for "optimum" growth in this series of cultures, since a reduction of this percentage from 0.25 to 0.15 depressed the total growth about 40 per cent, and the yield of grain (table 3) 58 per cent. The "sub-optimum" phosphoric acid soil cultures were perhaps a trifle below "optimum" growth conditions, as indicated by a 6 per cent growth depression. It may be estimated from these results that oats required 0.4 per cent phosphoric acid in dry matter to maintain "sub-optimum" growth when grown in the soil and one-half of this percentage when grown in solution. A much smaller phosphoric acid requirement (about 0.1 per cent) was found for the entire oat plant by Pember and McLean, based on the results of 5 years' experiments, so the results obtained here may have been exceptional.

The oats grown in the soil on both planes of potash nutrition, and those grown in the water cultures with a 580-mgm. application of this nutrient, show luxury consumption of potash. The 160-mgm. application of potash to the water cultures was decidedly less than the amount required. It can only be estimated, on the basis of these figures, that oats should contain in dry matter between 0.5 and 1.00 per cent of potash to insure the fulfillment of their requirements for this fertilizer nutrient.

SUPPLEMENTARY DETERMINATIONS

It was shown in tables 4 to 6, and it has been mentioned in the discussion of these tables, that the water cultures of barley, wheat and oats made a much greater growth per unit of absorbed phosphoric acid than did the soil cultures. With each cereal the phosphoric acid requirement for either "optimum" or "sub-optimum" growth in soil was approximately twice to three times the phosphoric acid requirement to fulfill similar conditions in solution. No explanation for this difference was found although further work was done in an effort to relate the reduced efficiency of phosphoric acid in the soil-culture cereals to the presence of precipitating metals.

Iron and aluminum in relation to phosphoric acid assimilation. It was thought possible to explain these differences on the basis of the idea advanced by Burgess and Pember (4): i.e. that a portion of the phosphoric acid absorbed by certain plants is of no nutritional value, but serves to precipitate absorbed aluminum. Cereals from both the soil-culture and water-culture series, which had received "optimum" and "sub-optimum" applications of phosphoric acid, were selected for analysis. The percentages of ferric and aluminum oxides which were found in these plants, as given in table 7, show that the amounts absorbed were very small. As might perhaps be expected, the pot-

grown cereals contained slightly larger percentages of aluminum oxide in most cases.

The percentages of iron and aluminum bore no constant relation to the percentages of phosphoric acid which were absorbed by the plants, nor were the amounts of these metals of sufficient magnitude to have interfered with the assimilation of this nutrient by combining with any considerable portion of it.

The soils in which Burgess and Pember found maximum aluminum toxicity with barley and lettuce possessed a degree of acidity corresponding to a pH value of 4.5. The reaction of the soil medium used in the pot cultures of the

TABLE 7

The percentages of ferric and aluminum oxides in the tops of plants grown in soil and in solution with "optimum" and "sub-optimum" phosphoric acid applications

CEREAL	CULTURE NUMBER		LIMITING CONDITION ASSUMED	Fe_2O_3 IN DRY MATTER OF THE TOPS		Al_2O_3 IN DRY MATTER OF THE TOPS		P_2O_5 IN DRY MATTER OF THE TOPS	
	Soil	Solution		Soil	Solution	Soil	Solution	Soil	Solution
				per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Barley	42	11	"Optimum" P_2O_5	0.055	0.039	0.021	0.013	0.63	0.32
	43	12		0.053	0.079	0.024	0.025	0.64	0.35
	52	5	"Sub-optimum" P_2O_5	0.057	0.042	0.017	0.010	0.47	0.17
	53	6		0.086	0.050	0.013	0.020	0.49	0.19
Wheat	60	43	"Optimum" P_2O_5	*	0.062	*	0.013	0.62	0.33
	61	44		0.078	0.032	0.003	0.016	0.55	0.30
	70	37	"Sub-optimum" P_2O_5	0.070	0.044	0.014	0.009	0.67	0.16
	71	38		0.086	0.039	0.022	0.015	0.62	0.20
Oats	78	27	"Optimum" P_2O_5	0.045	0.041	0.021	0.009	0.80	0.26
	79	28		0.063	0.045	0.017	0.005	0.74	0.25
	88	21	"Sub-optimum" P_2O_5	0.048	0.038	0.020	0.025	0.38	0.15
	89	22		0.064	0.046	0.000	0.015	0.44	0.16

* No sample available for the determination.

present experiment was not determined, but it seems certain, as previously stated, that it had a concentration of hydrogen ions equivalent to a pH value of between 6 and 7. These differences in reaction probably depressed the amount of "active" aluminum in the soil used for the pot cultures of this experiment.

Absorption of lime and magnesia in relation to the absorption of potash. Some plant physiologists have been inclined to the belief that calcium could in part replace potassium in the plant, when the latter element was deficient in amount.

TABLE 8

Amounts of calcium and magnesium oxides absorbed and the ratio of these oxides in the dry matter of the tops when plants were grown in soil and in solution with "optimum" and "sub-optimum" amounts of potash

CEREAL	CULTURE NUMBER		LIMITING CONDITION ASSUMED	CaO IN THE DRY MATTER OF THE TOPS		MgO IN THE DRY MATTER OF THE TOPS		AMOUNT OF CaO REMOVED IN THE TOPS OF THE PLANTS FROM EACH CULTURE		AMOUNT OF MgO REMOVED IN THE TOPS OF THE PLANTS FROM EACH CULTURE		AMOUNT K_2O REMOVED IN THE TOPS OF THE PLANTS FROM EACH CULTURE		RATIO OF CaO TO MgO IN THE DRY MATTER OF THE TOPS FROM EACH CULTURE	
	Soil	Solution		Soil	Solution	Soil	Solution	Soil	Solution	Soil	Solution	Soil	Solution	Soil	Solution
				per cent	*per cent*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*		
Barley	42	11	"Optimum" K_2O	0.34	0.74	0.38	0.42	0.147	0.320	0.164	0.181	0.669	0.630	0.9	1.8
	43	12		0.36	0.82	0.37	0.49	0.161	0.315	0.165	0.188	0.700	0.583	1.0	1.6
	40	15	"Sub-optimum" K_2O	0.37	1.15	0.41	0.57	0.150	0.360	0.173	0.178	0.430	0.284	0.9	2.0
	41	16		0.40	1.12	0.42	0.58	0.157	0.371	0.165	0.192	0.479	0.251	0.9	1.9
Wheat	60	43	"Optimum" K_2O	0.26	0.38	0.25	0.30	0.100	0.173	0.096	0.137	0.608	0.550	1.0	1.3
	61	44		0.24	0.37	0.22	0.28	0.101	0.174	0.093	0.132	0.620	0.540	1.1	1.3
	58	47	"Sub-optimum" K_2O	0.32	0.54	0.31	0.47	0.123	0.180	0.119	0.157	0.423	0.229	1.0	1.1
	59	48		0.26	0.55	0.31	0.43	0.102	0.194	0.122	0.151	0.453	0.225	0.8	1.3
Oats	78	27	"Optimum" K_2O	0.46	0.61	0.34	0.37	0.235	0.321	0.174	0.195	0.817	0.604	1.4	1.6
	79	28		0.41	0.53	0.34	0.32	0.227	0.301	0.188	0.181	0.853	0.584	1.2	1.7
	76	31	"Sub-optimum" K_2O	0.49	0.66	0.39	0.43	0.251	0.274	0.200	0.178	0.604	0.195	1.3	1.5
	77	32		0.48	0.73	0.35	0.42	0.237	0.317	0.152	0.182	0.602	0.203	1.6	1.7

Pfeffer (17) believed that this was possible. Magnesium, perhaps because of its universal association with calcium, has been similarly considered. Most of the experiments which have been carried out to test this point, however, have given negative results. F. Münter (13) in a series of field experiments with certain cereals found that neither nitrogen, phosphoric acid nor potash fertilization had any effect on the lime and magnesia content of barley. Hartwell et al (8) found in a series of water-culture experiments with wheat that no increase in growth resulted when a deficient application of potash was supplemented by additions of calcium sulfate solution.

It was not possible in this experiment to secure any data bearing on the actual replacement of potassium by calcium in the plant economy. An opportunity was offered simply to observe whether differences in the amounts of potash absorbed by the same cereal when it was grown on different planes of potash nutrition were accompanied by differences in the amounts of lime (CaO) and magnesia (MgO) taken up by the plants, and whether the latter amounts were roughly inversely proportional to the former. Plants representative of "optimum" and "sub-optimum" potash conditions from both the soil and the water cultures were analyzed for lime and magnesia. The resulting determinations as contained in table 8 do not show any clear relationship between the amount of potash in the plants and the intake of lime and magnesia, but the percentage of the latter two ingredients increase when potash is reduced.

The ratio of lime to magnesia in the plants analyzed for these oxides is given in table 8. The few results which are recorded show that the ratio varied from 0.8:1 to 2.0:1. The ratio appears to be higher with the plants which were grown in solution. No toxic effects were noted with the wheat in culture 59, although the ratio of lime to magnesia in the dry matter of these plants dropped to 0.8:1. The dry matter of all the soil cultures of barley contained more magnesia than lime, and the growth of this cereal was not depressed. The lime-magnesia ratio in the dry matter of the barley plants from culture 15 was 2:1, while this ratio was 1.1:1 in the dry matter of the wheat from culture 47. The amount of growth in both cultures was limited by a lack of potash, but there were no other indications of abnormality during the growth period.

The above results are in line with those of Wheeler and Hartwell (21), who showed that it was not necessary in moderate concentrations to maintain any definite lime-magnesia ratio to obtain the best growth of barley in pots. Their results show that the yield was not affected when this ratio, in the dry matter of the plants, varied from 0.6 to 1.9. The contention of Warthiadi (20) that it was necessary for the lime-magnesia ration to be 1:1 in cereal plants grown in both sand and water cultures, is not upheld by the data given in table 8 and mentioned in the preceding paragraphs.

THE RELATION OF SILICA TO THE ABSORPTION OF POTASH AND TO THE NUTRIENT REQUIREMENT FOR PHOSPHORIC ACID

Although silicon is classed as a non-essential element, it has been contended by some scientists that while silica is not necessary to plant growth, it nevertheless performs valuable functions in the physiological economy of certain species. This assumption has been made with regard to the general growth of cereals.

Pfeffer (17) believed that "silicon like calcium may help further to reduce the minimal amounts of phosphorus or potassium necessary for development."

TABLE 9

Silica and potash in the top of cereals grown in soil and in solution with "optimum" and "sub-optimum" potash applications

CEREAL	CULTURE NUMBER		LIMITING CONDITION ASSUMED	SILICA IN DRY MATTER OF THE TOPS IN EACH CULTURE		AMOUNT OF SILICA REMOVED BY THE TOPS IN EACH CULTURE		AMOUNT OF K_2O REMOVED BY THE TOPS IN EACH CULTURE	
	Soil	Solution		Soil	Solution	Soil	Solution	Soil	Solution
				per cent	*per cent*	*gm.*	*gm.*	*gm.*	*gm.*
Barley	42	11	"Optimum" K_2O	0.88	0.46	0.380	0.199	0.669	0.630
	43	12		0.86	0.55	0.384	0.211	0.700	0.583
	40	15	"Sub-optimum" K_2O	0.76	0.40	0.321	0.125	0 430	0.284
	41	16		0.84	0.37	0.330	0.122	0.479	0.251
Wheat	60	43	"Optimum" K_2O	0.90	0.85	0.347	0.389	0.608	0.550
	61	44		0.88	0.79	0.371	0.371	0.620	0.540
	58	47	"Sub-optimum" K_2O	0.92	0.65	0.354	0.216	0.423	0.229
	59	48		0.97	0.69	0.382	0.243	0.453	0.225
Oats	78	27	"Optimum" K_2O	0.81	0.63	0.414	0.331	0.817	0.604
	79	28		0.79	0.71	0.438	0.403	0.853	0.584
	76	31	"Sub-optimum" K_2O	1.56*	0.52		0.216	0.604	0.195
	77	32		0.81	0.55	0.400	0.239	0.602	0.203

* Sample apparently contaminated with soil.

Hall and Morison (6) have shown that yearly applications of sodium silicate to one of the field plots at the Rothamsted Experimental station have caused the barley grown on that plot to assimilate larger amounts of phosphoric acid. The "seat of action" of the silica is stated to be within the plant and not in the soil. Conner (5), however, believes that the beneficial results which these workers secured by the use of sodium silicate are due to the fact that this salt combines with soluble aluminum salts in the soil to form the

relatively insoluble compound, aluminum silicate, thereby eliminating the effects of aluminum toxicity.

The cereals from both the soil and water cultures which had received "optimum" and "sub-optimum" applications of potash were analyzed for silica, in order to determine whether a lesser absorption of potash was accompanied by a larger intake of silica. The results of these analyses comprise the major portion of the data of table 9, and show that the amounts of silica removed by the plants in most cases decreased with the smaller amounts of potash absorbed thereby. This decreased removal of silica was brought about not only by

TABLE 10

Comparison of growth and amount of phosphoric acid absorbed by certain cultures of cereals when potash was applied in chloride and in silicate

CEREAL	CULTURE NUMBER	TOTAL AMOUNT OF FERTILIZER NUTRIENTS ADDED PER CULTURE	WEIGHT OF STRAW PLUS GRAIN	WEIGHT OF STRAW	WEIGHT OF GRAIN	P_2O_5 IN DRY MATTER OF THE TOPS	K_2O IN DRY MATTER OF THE TOPS	SILICA IN DRY MATTER OF THE TOPS
		gm.	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*
Barley	5	0.656 N 0.050 P_2O_5 0.580 K_2O	31.3	22.6	8.7	0.17		
	6		38.4	26.3	12.1	0.18		
Wheat	37	Potash added mostly in potassium chloride	32.5	24.4	8.1	0.16		
	38		33.6	26.0	7.6	0.20		
Oats	21		35.8	27.6	8.2	0.15		
	22		35.4	27.0	8.4	0.16		
Barley	7	0.656 N 0.050 P_2O_5 0.580 K_2O	34.7	23.4	11.3	0.18	2.25	2.23
	8		33.3	22.2	11.1	0.09	2.28	1.71
Wheat	39	Potash added mostly in potassium silicate	34.2	26.0	8.2	0.13	2.06	2.49
	40		32.1	24.7	7.4	0.11	1.99	2.35
Oats	23		37.4	28.6	8.8	0.09	1.75	1.78
	24		37.9	28.6	9.3	0.11	1.84	1.49

smaller production of dry matter due to a deficient supply of potash, but by a smaller percentage of silica in this dry matter.

With the wheat which was grown in the soil cultures, however, the indications are that a decrease in the amount of potash taken up by the plants was accompanied by an increase in the amount of silica removed thereby. This increase in the amount of silica removed by the plants is not pronounced.

It was mentioned in the text that two of the water cultures of each series received potash mostly in potassium silicate. The data for the series of 6 cultures, which comprise table 10, are comparable with those which are re-

corded for "Sub-optimum P_2O_5" cultures in tables 4 to 6, and the latter figures have been included in table 10.

Reference to the table shows that the amounts of straw and grain produced by the barley, wheat and oats grown with potassium silicate as the main source of potash were practically the same as the weights of straw and grain from the barley, wheat and oats grown with potassium chloride as the main source of potash.

It is interesting to note that, with the exception of the barley grown in culture 7, the cereals in each of the cultures to which potash was applied mostly in potassium silicate absorbed less phosphoric acid than the cereals which were grown with equal applications of phosphoric acid but with potassium chloride rather than potassium silicate as the main source of potash.

Since the use of potassium silicate with a constant application of phosphoric acid did not increase the production of either grain or straw, it cannot be contended that silica usefully supplemented a deficient amount of phosphoric acid in either barley, wheat or oats.

However, it does not seem unreasonable to conclude that the use of a salt of potassium, which yielded potassium and silicate ions, resulted in a reduced absorption of phosphoric acid and no depression in growth of the plants grown in the nutrient solution which contained these ions.

The potassium silicate was rather completely absorbed by the plants in all 6 of the above cultures, as is shown by the amounts of potash and silica which they contained.

GENERAL DISCUSSION

The differences in the amounts of nitrogen and potash required by the cereals when grown in the different media were small, and the results show that in the case of these three cereals, at least, the conditions necessary for the absorption of these fertilizer nutrients were not different in soil and in solution.

The difference in the amounts of phosphoric acid required for the "optimum" growth of the cereals of the soil and of the water cultures was the principal point of dissimilarity between the growth of the three cereals in soil and in solution. While the reasons for the greater phosphoric acid-requirement of the pot-grown cereals cannot be stated, it was shown that the greater absorption of this nutrient by these plants was not due to the fact that it was utilized within the plants as a protective agent against aluminum or iron toxicity.

In the consideration of what constitutes "optimum" growth, the straw-grain ratio has been emphasized. This ratio is a factor of great economic importance and should not be overlooked. It was greater in the dry matter of the plants from the water-culture series than from the soil-culture series. The reasons for this cannot be stated.

SUMMARY

In this experiment, plants of barley, wheat and oats were grown to maturity in Wagner pots in the greenhouse, side by side with similar plants which were

grown to maturity in jars of nutrient solution. The medium used in the pots was made up of soil and sand.

Certain general aspects of growth of the plants in each medium were correlated, and the amounts of nitrogen, phosphoric acid and potash actually required by the plants for optimum growth in the soil and in solution were compared. The amounts of these mineral nutrients which were found to be necessary for the optimum growth of each cereal are expressed as per cent in dry matter of the tops.

It was found that the amount of nitrogen required by these three cereals in each medium was practically the same. The dry matter of "sub-optimum" cultures of barley in both soil and solution contained 0.9 per cent of nitrogen.

The data indicate that the percentage of nitrogen in the dry matter of wheat with "sub-optimum" growth conditions in soil was about 1.0, while in water-culture wheat this figure was between 0.8 and 1.0. The amount of potash required was probably not more than 1.1 per cent, and may have been less.

It was estimated that oats required only 0.6 to 0.7 per cent of nitrogen and between 0.5 and 1.0 per cent of potash in their dry matter, in order to make a slightly "sub-optimum" growth.

It was found that the dry matter of the water cultures of all three cereals which had made an estimated optimum growth, contained only about one-third to one-half as much phosphoric acid as the dry matter of the soil cultures of the cereals under similar conditions. Owing to the small number of cultures used and to the fact that the desired phosphoric acid conditions were not attained in all cases, the amounts of this nutrient required by the cereals grown in the soil could not be closely estimated. The required phosphoric acid did not exceed in any case 0.6 per cent in dry matter.

The cereals which were grown in soil yielded more grain per unit weight of straw than the cereals which were grown in solution. One factor which may have contributed to this difference: i.e., the difference in duration of concentration of nitrates in soil and in solution, is briefly discussed in the text.

The percentages of iron and aluminum in certain of the soil-grown plants were compared with the percentages of these elements in the plants from certain of the water cultures. Sufficient amounts of ferric and aluminum oxides were not present in either the soil-grown or solution-grown plants to interfere with the assimilation of phosphoric acid.

Slightly larger amounts of lime and magnesia were contained in certain of the cultures of cereals when the amounts of potash were small than when the amounts of potash were large. There was no connection between the ratio of lime to magnesia in the dry matter of the plants and the growth made.

The use in certain of the water cultures of a salt of potassium which yielded potassium and silicate ions resulted in a decreased absorption of a deficient amount of phosphoric acid, but in no decrease in growth.

ACKNOWLEDGMENT

In conclusion, the authors wish to extend thanks to Dr. Burt L. Hartwell for assistance in outlining the problem, and to recognize the value of timely suggestions and criticisms given by Dr. Paul S. Burgess and Dr. Forman T. McLean.

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SOIL MICROBIOLOGY IN 1924: AN ATTEMPT AT AN ANALYSIS AND A SYNTHESIS[1]

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Received for publication October 30, 1924

Devant une étape nouvelle une tendence se fait généralement sentir: celle de dresser un bilan des notions aquise, de se refraichir les idées, de combə̈tre les illusions qui s'implantent malgré nous dans notre esprit, tout cela pour bien choisir la direction des efforts et le programme du travail.—S. WINOGRADSKY (76).

INTRODUCTION

More than half a century has passed since Pasteur announced his ideas on the rôle of microörganisms in the decomposition and mineralization of organic matter. More than a quarter of a century has passed since Winogradsky and Beijerinck utilized the elective culture method for the isolation of the nitrifying and nitrogen-fixing bacteria from the soil. Still the science of soil microbiology is in its infancy, and one may rightfully ask whether such a science exists at all at the present time. While the study of microörganisms causing human, animal and plant diseases has made rapid progress, while the study of microörganisms concerned in the transformation of milk, in the ripening of cheese and in various industrial fermentations has brought forth a great deal of light upon these processes and greatly stimulated their development, soil microbiology has remained stationary. Since the development of the gelatin-plate method by R. Koch, the work of Hellriegel and Wilfarth on symbiotic nitrogen-fixation, and the classical discoveries by Winogradsky and Beijerinck of the nitrifying, nitrogen-fixing and sulfur-oxidizing bacteria, attention has been paid chiefly to working out various minor details. With the exception of the more proper utilization of leguminous plants, agricultural practice has hardly been modified as a result of the development of soil microbiology. A large number of microörganisms have been isolated and cultivated on artificial media, a great deal of information upon the physiology of these organisms has accumulated; but this belongs to the realm of pure microbiology and not to applied soil microbiology. The very existence of such a science is still questioned in many quarters. According to Winogradsky, the microbiologist has merely looked for organisms which he could attach to certain known processes, and has limited himself to that. He took no interest in the rest of the soil population. It was too complex for him and, therefore, non-existent. No attempt was even made to understand the mechanism of decomposition of the various organic substances added

[1] Paper No. 233 of the journal series, New Jersey Agricultural Experiment Stations Department of Soil Chemistry and Bacteriology.

to the soil. He limited himself to making ammonia and nitrate determinations, if the substance was nitrogenous in nature, or to measuring the evolution of carbon dioxide, if it was composed chiefly of carbohydrates. The complex mechanism of carbon-nitrogen relationships in the soil was little understood. Whenever a chemist approached the problem, he usually extracted the soil with acids or alkalies, treating it as though it were a dead organic and inorganic mass of substances and not full of dynamic processes, constantly modifying its composition. Still worse, he was always ready to draw practical conclusions and make broad generalizations from experiments whose foundation upon true soil processes could often be questioned.

The soil is a living medium, more or less colloidal in nature, containing a great mass of microscopic forms of life. These produce various physical and chemical changes in the soil which are of greatest importance to the growth of higher plants. The pathologist can study the action of his organisms *in vivo;* the microbiologist working on fermentation processes can sterilize his medium, without altering its composition greatly, and inoculate it with a pure culture of the organism concerned; the soil microbiologist, however, has great difficulties in attempting to learn just what the particular organism does in the soil. When the latter is sterilized, it is no longer, biologically and chemically, a normal soil. One may be led, then, to make a hasty, but wrong conclusion that the action of microörganisms in the soil is the same as in pure culture upon artificial culture media. In the latter case, free from stimulative and competitive influences of other microörganisms, an organism may manifest certain activities, which would not take place in the soil, or *vice versa.* It is even possible that, in pure culture, different races develop from those present originally in the soil and it is quite probable that the biochemical action is often quite distinctly different. As a matter of fact, a very large number of soil organisms develop on artificial media only with great difficulty and are often repressed there by other organisms which may be only occasional visitors in the soil. We do not know whether the activities of the organisms take place in the colloidal film or in the soil solution nor do we know to what extent one group follows another in producing a series of progressive changes in the composition of certain soil constituents.

The above considerations are chiefly responsible for the fact that a number of younger workers, recently very active in this field, have gone into other chiefly allied fields. When some of the older workers die (Hiltner, Vogel, A. Koch in Germany), their places are filled either by men working in entirely different fields, or by representatives of other agricultural sciences, such as chemistry or botany; very few new men come into the field. This is particularly evident in Germany, where the tendency seems to be to limit the subject of soil microbiology as much as possible, and in the United States, where some of the workers, very active ten to fifteen years ago, have found greater interest in fields of plant nutrition, soil surveying, industrial fermentation, medical bacteriology or commercial work.

The reasons for this phenomenon, in the case of a science which lies at the very foundation of our soil treatment, if not of our whole agricultural economy, cannot be accounted for by the fact that the practical application of the science is limited to legume inoculation; or that we already possess enough theoretical information which should be applied to practice; or that the soil represents a complex medium whose microörganic population carries on so many activities that any information obtained working with the soil itself is worthless. These conflicting considerations as well as the conflicting ideas as to what soil microbiology is and what it can accomplish, become evident to anyone who attempts to make a closer investigation of this phenomenon. "Nitrogen-fixation and all that sort of thing . . . . . " is a conception of the subject held by one of the older German workers in the field. "If you cannot isolate one organism and study its physiology in detail, you had better leave soil microbiology alone. I am sick of pottering with a lot of dirt and making only ammonia and nitrate determinations; that is not going to lead us anywhere . . . . ." is the opinion of an American worker recently very active in this field. "The loss of interest in soil bacteriological research in Germany is chiefly due to H. Fischer, who constantly criticized the Remy-Löhnis solution methods. . . . . " said Dr. F. Löhnis, whose work in bringing together the literature in soil microbiology has proved to be a distinct service to the science. "It was the solution method of Remy and Löhnis, as well as the work of Hiltner, Stoklasa and others on soil inoculation with non-legume bacteria that caused the loss of interest in the subject among the German workers," said a German investigator, whose interest in the subject can be traced for more than 20 years. Even the direct soil or beaker method used extensively in America, did not fare much better, and most of the investigators, who have spent much time and energy on this subject, have gone into other fields. We still find here and there a contribution to the subject of *ammonification*, *nitrification*, *denitrification* or *nitrogen-fixation* in the soil, but it is either the result of a new attempt of a younger worker who has not yet experienced the disappointments of the older investigators, or it is carried on by a soil chemist, who often thinks that he is advancing the science of soil microbiology. "We have as yet no science of soil microbiology proper, although we possess a great deal of information on various groups of soil microörganisms and what they can do when grown in artificial culture media," said S. Winogradsky, whose exact contributions to our understanding of certain soil microbiological processes can hardly be equalled.

These opinions and considerations evidently point to the need of an investigation into the condition of the science in the various countries. An attempt will be made to analyze the situation and, if possible, to answer whether this is due to the limitation of the science, to the limitation of the workers who were active in this field, to the lack of proper methods of attack, or to other causes. To carry on such a study is not an easy task and it has various

limitations, but if any light is thrown upon this very complicated subject, the author will feel that his time has not been spent in vain.

The sum total of our knowledge of soil microörganisms and their activities in the soil is made up of contributions from institutions where special laboratories exist for this purpose and where special workers devote all their time to the study of soil microbiological processes; also of contributions by investigators working in allied sciences, who become temporarily interested in a particular organism occurring in the soil, or a particular process, which advances our understanding of soil biological phenomena. Various botanists, especially those interested in cryptogamic botany like F. Cohn, Beijerinck, Oudemans, Wehmer, Lendner, Hagem, C. Thom, Bristol, and others, have contributed to our understanding of organisms occurring in the soil. The same may be said of zoologists, like Cobb, DeMan, Wolff, Kofoid, and lately Cutler, and of medical bacteriologists, like R. Koch and Fränkel. Various chemists, especially biological and soil chemists, have contributed to our understanding of soil biological processes, e.g., Pasteur, Winogradsky and others. It is important to note that some of the most important contributions to the subject have been made by investigators working not in soil, or agricultural laboratories, but in the general sciences. Therefore, in attempting to learn what advance is being made at the present time in the science in question, we must search not only the agricultural institutions, but also the various botanical and chemical laboratories, where such investigations may be carried out.

Eleven countries have been visited and the question has been discussed with workers in agricultural and purely scientific fields. The fourth international congress of Pedology in Rome also presented an opportunity of meeting workers from other countries which were not visited.

The author takes this opportunity of expressing his indebtedness to Dr. J. G. Lipman, Director of the New Jersey Agricultural Experiment Station for his constant interest in this matter and for making this investigation possible.

HISTORICAL

The origin and development of soil microbiology have their roots in empirical practice, in botanical sciences, especially bacteriology and mycology, and in chemical sciences, expecially soil chemistry. The practice of composting, especially the preparation of nitre heaps, and the practice of green manuring have called attention to some important biological principles in soil economy. The bacteriologist and the mycologist have developed methods for the isolation and cultivation of a large number of soil organisms. The chemist has developed methods of studying the activities of these organisms. The medical and agricultural bacteriologists have contributed to the science by developing methods for counting various groups of organisms, for staining and otherwise studying the morphology and physiology of the organisms.

The greatest credit, in this connection, is due to S. Winogradsky and M. W. Beijerinck.

By the end of the nineteenth century, or more exactly about 1903–4, the most important fundamental biological processes in the soil, as far as our present knowledge is concerned, had been established; the various organisms had been isolated and described. Here belong the processes of oxidation of ammonia and nitrites, the fixation of atmospheric nitrogen by symbiotic and non-symbiotic bacteria, the reduction of nitrates, the oxidation of sulfur and its compounds, also, to some extent, the decomposition of organic matter, including proteins and celluloses. However, the exact nature of the latter process in the soil is not fully clear as yet, especially in respect to the formation of the complex mass of organic matter in the soil commonly known as "humus."

The development of the science of soil microbiology was then at its height. Great expectations arose everywhere. The brilliant contributions of medical bacteriology to human health and hygiene, of dairy and water bacteriology, of the microbiology of brewing and other fermentation processes, had led the practical agriculturist to expect similar results from soil microbiology. It was expected that, once the foundations of the science were laid, the further scientific development and, of course, the practical application would naturally follow. As Maaszen and Behn (43) expressed it, "one believed that it was time to devote special attention to the bacteria of the soil; the practical agriculturists believed that far-reaching explanations of the practical agricultural problems and great practical applications would result. It was due also to a large extent to practical people that numerous bacteriological investigations of the soil were undertaken with great vigor."

These high expectations were, however, not fulfilled. Outside of the practice of soil inoculation with legume bacteria and a proper understanding of the process of nitrification, few other practical applications resulted. Among these, we should mention the utilization of green manures, use of lime, of basic and acid fertilizers, tillage and soil disinfection. The further scientific development of the subject did not seem to expand, but rather to contract. It is interesting to note that the two classical workers in the field had practically ceased their activities at this period. New men have come in, but very few new ideas have come forth. Various isolated phenomena are now considered from different angles, such as cellulose decomposition, formation of ammonia from proteins, and denitrification, but most of these processes are still unexplained.

Two distinct lines of investigation can be noted at this stage:

1. The development of the plate and other methods for counting the numbers of microorganisms in the soil; here belongs the work of Hiltner and Störmer, H. Fischer, J. G. Lipman and P. E. Brown, H. J. Conn, and others. A number of these investigators have also worked on the isolation, in pure culture, and on the description of various bacteria concerned in certain physiological soil processes. These latter studies tended to produce a better knowledge of various groups of soil microörganisms, but they consumed a great deal of time and were

followed by few practical results. We need only mention here the names of A. Meyer and his pupils in Germany, and Chester and others in America.

2. The transformation of organic and inorganic substances in solution, inoculated with soil, or in soil to which certain specific substances were added; it is sufficient to mention, in this connection, the names of Remy, Löhnis, Vogel, Stoklasa, J. G. Lipman, P. E. Brown, C. B. Lipman, Christensen, and numerous others.

Probably these two groups of investigations would finally lead to a proper understanding of the soil biological processes. A great deal of important information has been accumulated, but no great scientific discoveries (outside of some important additions to our knowledge of certain groups of soil bacteria) have been made, nor did any practical results follow. These methods of attack resulted, however, in an almost entire loss of interest in the subject, both from the scientific and practical men. This is confirmed by the comparatively small number of contributions appearing at present in this connection. The trust in this kind of work is so much lost that, in the words of a younger German soil chemist, "to call oneself a soil bacteriologist at the present time is to ruin entirely one's chances for an advance in position in the future."

Several distinct contributions to the science of soil microbiology can be traced, however, to this period, especially on the microörganisms non-bacterial in nature. It is sufficient to mention the work of Sir John Russell and his associates on the occurrence of protozoa in the soil and on the phenomenon of partial sterilization; the occurrence and activities of algae, fungi and actinomycetes in the soil; the direct examination of microörganisms in the soil by H. J. Conn; a better understanding of the organisms concerned in the oxidation of sulfur in the soil; microörganisms concerned in the decomposition of cellulose in the soil; the influence of soil reaction upon the distribution and activities of soil microörganisms. All of these are, however, isolated phenomena and they have remained, in most instances, unconnected. No attempt has yet been made to bring all this information together and to develop proper methods for bringing soil microbiology into the position of an independent science. This becomes especially clear when one peruses the pages of the "Handbuch der landwirtschaftlichen Bakteriologie" by Dr. F. Löhnis: a large number of investigations, some of them very important, but without any centralized guiding idea of an existing science.

It remains to be seen now whether the present interest in the subject justifies the discouraging conclusions mentioned above.

ENGLAND

The *Rothamsted Agricultural Experimental Station* is the outstanding institution, not only in England but perhaps in all of Europe, where soil microbiological problems are considered from various angles and in great detail. An attempt is also made to consider and interpret the interrelationships between the different groups of microörganisms making up the soil population.

Not only the bacteria and the fungi, but also the algae and protozoa of the soil are investigated and some outstanding contributions to our knowledge of these groups of organisms, especially the last two, have been made. The work is carried on in different departments, named after the groups of organisms which are particularly investigated. An attempt is made to utilize the results of the various investigations for practical purposes, as the treatment of greenhouse and other sick soils with steam and various antiseptics, and the preparation of artificial manure.

The investigations carried on in the department of bacteriology by Thornton, Gray and others include the study of organisms concerned in the decomposition of phenol and its derivatives in the soil, the development of the plate method for counting bacteria, the introduction of new leguminous plants (Danish lucerne) and an investigation of the process of staining bacteria for flagella. The bacteria concerned in the decomposition of phenol in the soil are divided into three groups: *a.* Mycobacteria, which are capable of decomposing phenol, cresol and naphthol; 70 strains have been isolated, 3 of which also attack toluol. *b.* Pseudomonads, acting upon phenol and naphthol, but doubtfully upon cresol; 70 strains of these organisms as well have been isolated, some being related to *B. fluorescens*. *c.* Aerobic Clostridia acting only upon phenol and unable to fix any atmospheric nitrogen.

The investigations on the algae of the soil have definitely disposed of the theory that algae are able to fix any atmospheric nitrogen. This was shown to be negative for a series of grass-green algae by Roach and Page (10). Roach (Miss Bristol) also studies the occurrence of algae in the soil, the influence of environmental conditions on the development of algae and their nutrition in pure culture.

The study of soil fungi carried on by Brierley resulted only in a preliminary survey of the filamentous forms occurring in the soil.

The protozoological laboratory, in charge of Cutler, who is assisted by Sandon and Cowie, has brought forth some very interesting contributions to the numbers, types and activities of protozoa in the soil. Attention may be called here to only a few of the problems and some of the more important results recently obtained.

1. Method of determining the total numbers of protozoa in the soil. Twenty grams of soil are shaken with 200 or 250 cc. of water. A series of dilutions is prepared, usually 1:2 or 1:4, using 10-cc. pipettes and 30-cc. portions of sterile water. One-cubic centimeter portions of all the dilutions are placed in Petri dishes (2 for each dilution); the dishes contain cooled, solidified, sterile nutrient agar. The plates are then incubated at 18 to 20°C. and examined twice, after 14 and 28 days. The examination is made by adding a little sterile water to the surface of the agar, then scraping off with a loop from the surface of the agar a little of the growth, placing it on a slide and examining it under the microscope. The number of protozoa is obtained from the number of positive and negative plates, using a definite formula prepared by Fischer.

2. Determination of the number of living forms and cysts of protozoa in the soil. Five cubic centimeters of concentrated HCl is added to the original suspension of soil (20 gm.) in the

water (250 cc.); the mixture is allowed to stand over night, is then neutralized with NaOH and the number of protozoa again determined by making various dilutions and examining as before. The figures thus obtained give the number of protozoan cysts in the soil. The number of active forms is obtained by subtracting the number of cysts from the total number of protozoa.

3. Growth rate of protozoa and bacteria. A careful study of the numbers of protozoa and bacteria in the soil revealed the fact that the changes in the numbers of bacteria are conditioned by the changes in the numbers of protozoa in the soil; there was no evident correlation between the protozoa and the external conditions. Reproduction rate of protozoa is the only index of development of these organisms; the number of divisions *Colpidium colpoda* undergoes in definite periods of time is intimately connected with the size of the bacterial population (18).

4. Influence of protozoa upon the development and activities of bacteria. Cutler (17) found that the addition of protozoan cultures (not free from bacteria) to cultures of bacteria in sand media resulted in a diminution of the number of the latter. This is in line with our knowledge that protozoa feed upon bacteria. This does not tell, however, that the destructive effect is injurious to the microbiological processes. In this connection the experiments of the Hindu bacteriologist Nasir (50), working at Rothamsted, are very instructive. Nasir found that the presence of protozoa in Azotobacter cultures brought about, in the majority of cases, an increase in the amount of nitrogen fixed in the culture. The nitrogen is fixed by the Azotobacter cells in the process of their development. The protozoa destroy the Azotobacter cells, thus bringing about a further development of the latter and thus also a further fixation of nitrogen. Since this may be true of various other bacterial processes, we need not conclude that the destruction of bacteria by protozoa proves to be injurious to soil fertility which is dependent on microbiological processes. The activities of protozoa in the soil may prove, as in the experiment of Nasir, not only uninjurious, but decidedly beneficial. By destroying the excess of living or dead bacterial cells, the protozoa, although bringing about a temporary reduction in the numbers of bacteria, may stimulate a further increase of bacteria, thus bringing about a stimulation of the biochemical processes in which the particular bacteria are concerned, since the biochemical process is chiefly a result of the multiplication of the bacteria. On examining a series of soils collected from different parts of the United States, Allison (1a) found that the important groups of protozoa are uniformly distributed in these soils, similar to English soils, but they differ quantitatively, the protozoa occurring to a more limited extent in the American soils.

Russell and Hutchinson originally considered the protozoa to be the factor limiting the development and activities of bacteria in the soil. This theory has stimulated numerous fruitful researches, not only on the soil protozoa, but on the interrelations of different groups of microörganisms in the soil in general. The original position of Russell and Hutchinson is now greatly modified. The tendency is to emphasize that the protozoa are *one* of *the* important factors which control the complexity of the microbiological processes in the soil and which maintain the equilibrium of the soil population.

We must also mention the numerous contributions of the Rothamsted Station, coming from various departments, on the influence of heat, steam, and volatile and non-volatile antiseptics upon microbiological processes and upon the fertility of the soil. These were originally intended to throw light upon the rôle of protozoa in the soil. It has not been as yet definitely proven whether the mere killing of the protozoa may not contribute to the beneficial action of the disinfectants, due to the increase of available organic

matter in the soil. These studies resulted in a great deal of practical information on the treatment of greenhouse and other sick soils. They have not, however, fully disentangled the fact as to what phenomena soil sickness is due.

The investigations of Page, the chemist of the station, and of his associates, on the composition of soil organic compounds, are also of great interest to our understanding of certain biochemical soil processes. Soil taken from heavily manured and unmanured plots was extracted with cold 2 per cent NaOH solution; the total carbon was determined in the extracts. The ratio between the carbon content of the manured and the unmanured soils was found to be 23 to 8; when the carbon contents of the consecutive extracts are plotted, the curves obtained for the two soils are the same; similar results were obtained when hot NaOH solutions are used. These results tend to indicate that, although the content of organic matter in the soil may be different, the nature of this organic matter is the same. Some factors active in the soil seem to lead to the establishment of a certain kind of organic matter, the same in composition and independent of its amount and of its original nature. Microörganisms no doubt play an important part in this connection.

The formation of the so-called humus and humic acids in the soil is now explained by four different theories: (*a*) the formation of humic acids from lignins, (*b*) from furfurol, (*c*) from amino acids interacting with carbohydrates, (*d*) as a result of oxidation of phenylic substances. According to Page, cellulose is not responsible for the formation of humic acid; lignin is, however, oxidized to humus; artificial humic acid can be prepared from lignin by treating it, at ice temperature, with 41 per cent HCl. Proteins probably play an indirect part in the formation of humic substances. Since pentoses give artificial humic substances much more readily than hexoses, it is believed that pentosans may play a part in the formation of the complex soil organic matter commonly called "humus."

An attempt at utilizing the activities of microörganisms for practical purposes is made by Richards, who is working on the preparation of an artificial manure. The process consists in cutting up straw and forming piles of this material moistened with an aqueous solution of potassium and nitrogen salts (ammonium salts preferred), as well as phosphates. The piles are kept well moistened. The various microörganisms developing in the piles can utilize the carbohydrates of the straw as a source of energy, which enables them to assimilate the inorganic nitrogenous compounds and minerals and synthesize organic material. The whole mass becomes transformed (in about 3 months) into a mixture of undecomposed lignins, partially decomposed other organic substances, and synthesized microbial cells, which consist of proteins, carbohydrates and fats. The final product contains about 2.0 to 2.5 per cent nitrogen, 1.2 per cent P_2O_5 and a variable amount of K_2O. In view of the fact that the final product is still rich in cellulose, it is suggested that various bacteria (like *B. mycoides*) and fungi decompose the starches and pentosans

of the straw and a small amount of the cellulose; this source of energy enables the organisms to assimilate the ammoniacal nitrogen and transform it into protein nitrogen. The lignins and the larger part of the celluloses remain in the heap undecomposed. This accounts for the high carbon-nitrogen ratio (20:1) of the product, since the fungi, which are concerned largely in the decomposition of cellulose in the soil, act much better under well-aerated soil conditions. It may be added that not only the chemistry of this particular process of composting remains to be investigated, but an accurate knowledge of the organisms concerned is also lacking. It is claimed that this artificial manure has been used successfully in various counties in England.

In summarizing the work carried on at Rothamsted, we find that the investigations are directed toward giving us a proper idea of the soil population, course of changes of this population, especially throughout the year, and the correlation between the members of its different constituent groups. The bacteria are not the only members of the soil population concerned in the fertility of the soil; the fungi, algae and protozoa occur in great abundance and play an important part in the biochemical processes in the soil. Since nitrogen is the controlling element in the growth of cultivated plants, and since microörganisms take an active part in the transformation of this element in the soil, the various processes should be studied in detail, so that we may learn to understand and control them.

No other laboratories in England have special departments of soil microbiology and only a few devote any considerable time to this field of investigation. We need only mention the work of Millard (49) at Leeds on the soil actinomycetes, especially in connection with the development of *Act. scabies* in the soil, of Thompson (68*c*) at Aberystwyth on the non-protozoan fauna of the soil, of Rayner and his associates, at Bedford College, on mycorrhiza fungi, and the recent contributions of Whittles (73) at Cambridge on the determination of the total number of bacteria in the soil. The latter developed an apparatus whereby a certain number of vibrations per minute are imparted to the soil suspension. By using this method of dispersion, the numbers of bacteria found in the soil were made more comparable to those obtained in direct counts.

It may also be of interest in this connection to call attention to some of the recent contributions to soil microbiology from the British colonies. Norris and associates (52) at India (Pusa Agricultural Research Institute) have contributed to our understanding of the decomposition of calcium cyanamide in soils. Meggitt (48) has also published recently a study on the nitrogen cycle in an acid Indian soil and on the biological activities, as measured by the evolution of carbon dioxide.

Hall (33) in South Africa found that drying of the soil in winter and the action of frost seem to improve the nitrifying power of the soil, when conditions become favorable to nitrification; moisture and aeration generally influence nitrification in South African soils more than temperature.

The investigations carried on by Lochhead (42), at the Central Experiment Station, Canada, lead us to dismiss finally the idea presented by Conn and confirmed by Brown that a distinct bacterial flora exists in frozen soils in the winter different from that of the summer flora. The large increase in the number of bacteria was obtained only at the end of the frost period with the thawing of the soil. No typical winter flora could be found; the bacteria of frozen soil are to be regarded rather as cold-enduring than psychophilic in the true sense.

FRANCE

The soil microbiological investigations carried on in France can be summarized under the following headings: (*a*) The investigations of S. Winogradsky in the newly organized division of agricultural microbiology of the Pasteur Institute. (*b*) Investigations carried on in the other sections of the institute, largely in the section of biological chemistry. (*c*) Investigations carried on in the Institute Agronomique and in the Institute Recherche Agronomique. (*d*) Research in the commercial laboratory of G. Truffaut. (*e*) Investigations in other French laboratories.

In a typical small French town, Brie-Comte-Robert, about 20 to 25 miles from Paris, in a little side street (Rue Pasteur), the visitor will find, after walking along a high, white-plastered fence, a small tablet near a large door. The tablet bears an inscription:

INSTITUTE PASTEUR

MICROBIOLOGIE AGRICOLE

Beyond this fence, lives and works S. Winogradsky, the man who has produced more than anyone else, the classic contributions to soil microbiology. His work was not so many-sided as that of Beijerinck, but it was as exact as it can ever be made. It is worth while to consider briefly, in this connection, the life and contributions of this investigator to soil science.

Sergei Nikolaevitch Winogradsky was born in Kiev in 1856. His father was a wealthy banker and "pomieshtshik" (landowner), who possessed large land estates in the Ukraina, namely, in the Podol region, near Proskurov. Sergei Nikolaevich received his early education in the schools of Kiev. After graduating from the gymnasium in 1872, he entered the University of Kiev and studied there for two years. At first he took up law, but then changed over to the study of natural sciences. At the end of two years he transferred to the University of St. Petersburg where he made a special study of plant physiology, under the direction of the famous Russian botanist, Famenzev. At the same time he became greatly interested in music, and took lessons in piano with the well-known Lieshetizky. The young Winogradsky became a virtuoso both on the piano and violin-cello. In 1877 he completed his work at the university and was left there as a special student with Famenzev, under whom he received the degree of Master of Science in Botany. At the

end of his training in St. Petersburg, Winogradsky left for Strassburg, where he entered the laboratory of De Bary; he also took special work, at the same university, in chemistry. When De Bary died he left for Zürich, where he continued his chemical studies under Ernst Schultze, and carried on independent investigations at the Hygienic laboratory.

The investigations on the sulfur and iron bacteria were carried on in Strassburg. The "Beiträge zur Physiologie und Morphologie der Bakterien" were also published there. The work on the nitrifying bacteria was begun in Zürich and the first paper on this subject appeared in 1890. At that time, Ilia Ilitch Metchnikov came to Zürich and invited Winogradsky, in the name of Pasteur, to come to Paris and establish a section of soil microbiology at the Institute Pasteur. However, at the same time, Prince Oldenburg, President of the Institute of Experimental Medicine, invited Winogradsky to come to St. Petersburg. Winogradsky decided to accept the Russian invitation and return to his homeland, otherwise he was afraid that he would have to be away from his home all the time. On his way to St. Petersburg, Winogradsky went to Paris and met Pasteur, who was at that time old and paralyzed. In 1891, Winogradsky entered the service of the Institute of Experimental Medicine, as chief of the Division of Microbiology, and in 1904 he was made Director of the Institute. He also edited the Archives des Sciences Biologiques.

The increased political disturbances, as a result of the Russo-Japanese war, the dissatisfaction with the pure administrative duties at the institute, ill-health, and the fact that he was economically independent, made Winogradsky give up his position in 1907 and retire to private life, remaining honorary member of the institute. Thereafter he lived mostly abroad (in Switzerland) and part of the time on his estates in Podol. The investigations on the nitrifying bacteria were finished in St. Petersburg; his studies on Clostridium, the non-symbiotic nitrogen-fixing organism, were begun and finished there. The two outstanding pupils of Winogradsky were V. L. Omelianski and Fribes, the latter having made his contribution to the process of retting of flax.

The latest revolution in Russia forced Winogradsky to leave his homeland; a French warship carried him away from Odessa. He found himself in Switzerland, with very little means to live upon. The Jugo-Slav government then extended to him the invitation to come and organize a department of microbiology in Belgrade. This proved, however, to be a rather unpleasant task under the existing conditions. The Institute Pasteur at Paris again extended in 1922 the invitation of 30 years previously, to come and organize a section of soil microbiology. Winogradsky gladly accepted.

Winogradsky is particularly interested at present in finding out which microörganisms live normally in the soil and which develop there as a result of addition of specific organic and inorganic substances or other changes of soil conditions. For this purpose the direct examination of soil, originally

suggested by H. J. Conn at Geneva (N. Y.), has been utilized and developed further (75). A suspension of the soil in water is either placed directly on a slide or is first centrifuged for different periods of time, then the suspension or the sediment is placed upon slides. The smear is allowed to dry upon the slide. To prevent the removal of the coarser particles, 1 per cent hot or 0.1 per cent cold agar solution is employed. The preparation is then fixed for from 1 to 2 minutes in absolute alcohol; after the alcohol is evaporated, the preparation is stained. For this purpose acid dyes are employed. Erythrosine was found to be best (rose-bengal tends to over-color; acetic acid may then be used to decolorize, the color returning on drying). About 1 per cent of the dye is dissolved in a 5 per cent phenol solution. The dye is allowed to act for from 5 to 15 minutes and is then washed off with water. The dye distinguishes the bodies of the microörganisms from the gelatinous capsules which surround the colonies and from the colloidal and other soil particles. The organisms are always found in colonies upon the soil colloids, but not upon the mineral particles directly nor in the soil solution.

Winogradsky's idea (74) at the present time is that a normal soil contains an aboriginal (autochtonous) flora, which consists entirely of cocci (grouped in colonies or zooglea) and non-spore forming bacteria; to some extent of actinomycetes and of fungus spores. Protozoa are never observed. When an organic substance or nitrogen source is added to the soil, a change in the flora takes place: the spore-forming bacteria and fungi become active. Winogradsky thus differentiates between the *microbiological*, or simply biological, *state* of the soil, including the quantity and quality of microbial cells which it contains; and the *biological reaction* of the soil, or the changes produced as a result of the addition of various substances. For this purpose, the original soil (control soil) and the treated soil (cultured soil) are studied side by side. In addition to the direct microscopic examination, auxiliary cultures can also be employed, by placing solid agar or silica gel media, corresponding in their composition to the soil as much as possible, in Petri dishes, and inoculating either with minute particles of soil or with different suspensions. No liquid media should be employed.

On adding a small amount of peptone to the control soil and incubating over night at 30°, the soil, originally found by direct examination to be free from spore-forming bacteria, now contains many millions of bacteria, representing only 2 species of bacilli. The change is explosive, both in kind and in degree; after 30 hours, the chemical reaction is completed and the bacteria change into the inactive spore state. The addition of urea also stimulates the development of two species of bacilli, while the addition of starch brings about an extensive development of one bacillus and of Actinomyces mycelium. Pentose stimulates the development of other organisms; cellulose brings about an extensive development of fungi. On adding dextrose or mannite to the soil, an extensive development of Azotobacter takes place, the number of cells reaching 350 millions per gram of soil; when the soil moisture is so high as to

result in submersion of the soil, the development of Clostridium takes place. However, when in addition to the mannite, available nitrogen (nitrate) is added to the soil, the development of Azotobacter diminishes, reaching 0, when 7 parts of nitrogen are added per 1000 parts of mannite; at this point another minute bacterium develops in great abundance; at different nitrogen concentrations other bacteria predominate. A substance favorable to the development of one group of organisms may become injurious when it favors another group.

These processes take place in succession with great rapidity. The soil is thus looked upon as a medium harboring all imaginable microörganisms. The great majority of these are usually latent in a normal soil, only a few remaining active. The latent organisms become rapidly active, when specific substances are added to the soil. The microbial processes consist of a multitude of phases, which are superimposed, each connected with the action of a single microbial agent or group of agents. The final phase brings about the establishment of the original aboriginal soil flora. To understand the activity of microörganisms in the soil, the microbiologist must understand these phases. Not only static microbiology, but also dynamic microbiology should be studied and understood. *Only when this is understood, shall we be able to speak of soil microbiology as an independent science, whose importance for agriculture cannot be overestimated* (76).

In addition to these studies, Winogradsky is also working at present upon direct methods for the isolation of different groups of soil microörganisms. For this purpose, silica gel media, to which a specific nutrient is added, are employed. When such plates are inoculated with minute particles of soil, the specific organisms will develop immediately, forming spreading colonies around the soil particles. Nitrite-forming bacteria will develop on the plate, when ammonium salts are employed as the only source of energy; nitrate organisms, when nitrites are used; Azotobacter, when mannite without nitrogen is employed.

As to the other contributions to soil microbiology made recently at the Institute Pasteur, we must call attention to the studies of Khouvine (38), working under Bertrand, on the isolation of a cellulose-decomposing bacterium from the intestinal tract of man. *Bac. cellulosae dissolvens* was cultivated on a medium containing peptone, K_2HPO_4, some $CaCO_3$ to neutralize the acidity, and some fecal extract. A good vacuum is required. The spores of the organism will survive a long time. This organism was isolated in 60 cases out of 100. In general, it can be cultivated on a medium containing cellulose as the only source of energy, and low degradation products of nitrogen, such as fecal matter, as sources of nitrogen. Among the products of degradation of cellulose, there were found CO_2, H_2, C_2H_5OH, acetic and butyric acids and a brown pigment—these forming only 60 per cent of the cellulose decomposed. Lactic acid and hydrolytic products precipitated with alcohol could also be demonstrated. It is believed that there are also formed saccharides soluble

in alcohol. In pure culture 1 gm. of cellulose is decomposed in 16 days, while 5 times as much is decomposed when the organism is accompanied by other bacteria.

Mazé, who has previously made some important contributions to the science, has recently carried on some investigations on legume inoculation but could obtain no practical results in the case of the common leguminous plants, at least as far as France is concerned.

At the Institute Agronomique, Guittonneau (30) is working on the physiology of certain actinomycetes, which were found capable of forming urea from peptone. It is, therefore, suggested that these organisms form urea as an intermediary product in the decomposition of proteins. It is rather unfortunate that the term "Microsiphonales" has been applied to the Actinomyces group, already overburdened with more names than most other groups of microörganisms. François is studying, at the same institute, the occurrence and activities of protozoa in the soil.

Bruno (12), of the Ministry of Agriculture, mentions the following investigations, dealing with soil microbiological problems, carried on in different laboratories in France: Boulanger at Lille, Dupont at Nancy, and Rivière at Versailles working on partial sterilization of soil, the activities of the bacteria being measured (Dupont) by determination of numbers, evolution of CO_2, and nitrifying capacity. Guittonneau at Paris working on nitrogen fixation; Demolon at Laon, on sulfur oxidation; François at Paris, on the activities of protozoa in the soil; and Kayser at Paris, on general soil microbiology.

At the Institute of Applied Chemistry, Schlösing *fils* is carrying on investigations on the drying of soils, which may prove of great interest to soil microbiology. It must be remembered that this investigator was the first to establish the interchange of gases in the fixation of nitrogen by leguminous plants. The work of Terroine and associates (68*b*), at the University of Strassbourg, on the energy transformation in the growth of fungi, using carbohydrates and proteins as sources of energy, will add valuable information to our understanding of the transformation of the soil organic matter.

Before finishing the review of the present contributions to soil microbiology made in France (in the laboratories visited), we must say a few words about the investigations carried on in the laboratory of G. Truffaut (70) in Versailles. Several investigators (notably N. Bezssonoff) were found to devote part or all of their time to soil studies. However, as far as soil microbiology is concerned, the investigations are largely along lines followed in other laboratories, notably in Rothamsted. Attempts are made to utilize these investigations for practical purposes.

The product Sulgine, containing CaS as a base, which has been used for the partial sterilization of soil, is now replaced by another one, containing calcium hypochlorite as a base. But it is recognized even in this laboratory that the practial utilization of soil antiseptics could not go further, at the present time, than in the case of greenhouse soils. Field soils do not justify, for the present

at least, such an application. Von Feilitzen and Barthel (24) tried the influence of Sulgine, as well as the so-called "disinfectant fertilizer Biogine," upon different soils and found these materials to be ineffective when used in quantities which would prove sufficiently inexpensive for practical purposes.

In the study of nitrogen fixation by *Clostridium pasteurianum*, Truffaut and Bezssonoff found that the use of a low concentration of sugar (0.1 per cent) proves to be more economical than the 1 per cent usually employed.

The present contributions to soil microbiology made in France are largely limited to those of Winogradsky. It is the hope, however, of some of the leading soil men in France that the investigations so successfully begun by Boussingault, Berthelot, Schlösing, Müntz, and others will be taken up again, as soon as properly trained men are available. According to Demoussy, the biological chemist is afraid of soil biology, one of the most complex of biological sciences, and would rather devote his attention to plant physiology or other sciences, presenting much simpler problems.

GERMANY

England can boast of a large institution in which the soil is studied from the physical, chemical, and biological viewpoints; four distinct departments of this institution are devoted to the study of the occurrence and activities of various groups of microörganisms in the soil. France can point to at least one laboratory, now an integral part of its largest biological and chemical institute, in charge of a man who was one of the first to lay the foundation of the science of soil microbiology. Germany, the country which perhaps has made a greater number of contributions to the science of soil microbiology than the rest of Eruope taken together, and where the science seemed about to reach its highest development, cannot point, at the present time, to any one investigator, who devotes his entire time to this subject. The reasons for this have been given above. However, a great many investigations dealing with soil microörganisms and with soil biological processes are carried on in the various universities and scientific laboratories throughout the country. These are done mostly by assistants, or form one of three or four topics under the supervision of one investigator, or are the subjects of doctorate theses. The future advance of soil microbiology depends upon the advances made in the fundamental sciences, notably physical and biological chemistry, as well as upon our understanding of the physical and chemical soil conditions. The modern investigator in this science has to be trained not only in bacteriology, but in mycology and protozoology, not only in chemistry, but also in the various special branches of chemistry, such as organic and physical. Soil microbiology has to depend, for its present advance, upon the work of students and beginners.

As mentioned above, Hiltner, Vogel, and A. Koch are dead; Löhnis is in America; O. Rahn and H. Fischer could not find suitable positions where they could carry on studies in soil microbiology, and have gone into

other fields of endeavor; H. Pringsheim devotes his time entirely to organic chemistry; Lemmermann is interested chiefly in purely chemical processes in the soil; Remy is interested in fertilization and plant growth. It is left to the pure physiologists, such as Ruhland in Leipzig and Meyerhof in Keil (now in Berlin); to some of the botanists, like Rippel in Göttingen and R. Falk in Münden; to some of the soil chemists, like Ehrenberg; and to general agricultural bacteriologists to make some of the important contributions to the subject. The present lack of funds for scientific research in Germany has led the investigators still at work to carry on as inexpensive experiments as possible. Various institutions were visited in Berlin, München, Leipzig Halle and Dresden; and the Congress at Rome presented an opportunity to discuss this subject with representatives from other institutions not visited. Still this review cannot lay claim to completeness, but gives a fair idea of the present condition of the science in Germany.

Seiser, at the Biologisches Versuchs Anstalt of the Bureau of Fischeries in München, has been devoting recently a great deal of attention to soil bacteriological processes taking place largely in river, soil, and mud. Among the various problems, in which he is interested at present, we may mention:

1. Symbiotic nitrogen-fixation by algae and bacteria; the work is at present carried on with crude cultures, but it will be later continued with pure cultures.
2. Nitrate transformation in the soil, including complete denitrification, partial reduction, and transformation of nitrates into proteins, as influenced by ammonium salts and phosphates; also a study of the influence of oxygen tension upon nitrate reduction in the soil.
3. Influence of colloids upon bacterial processes; at first nitrogen-free charcoal was used, next humic acids and their salts will be employed.
4. Influence of bacteria upon the transformation of insoluble iron phosphates into soluble salts, in the presence of sodium citrate as a source of energy.

At the Bavarian Peat Institute in München, founded in 1905 by Baumann, no attention is paid to microbiological investigations; even the chemical research is reduced to mere routine work; the prevailing idea is that we know enough of theory as applied to soil science and it is time to apply it to practice.

At the Hiltner Institute für Pflanzenbau und Pflanzenschutz, Kronberger, old time assistant of Hiltner, is still working on the development of certain non-nitrogen fixing bacteria, which stimulate the growth of non-leguminous plants, such as beets (34). The work is carried on with crude cultures, but it is claimed that some favorable results are obtained. Just what these bacteria do in the soil could not be definitely ascertained, except that they supply energy (sic.!) to the nitrogen-fixing bacteria in the soil. Baumgärtel, a pupil of A. Koch, gives the lectures in agricultural bacteriology at the Technological Institute. He is interested in the transformation of insoluble phosphates into soluble forms, in the composting of manure and generally in the decomposition of organic matter. It is hoped that he may take up again the work in soil microbiology which was, at the death of Hiltner, practically discontinued at the former institute.

At the Versuchs Anstalt in Dresden, two biologists interested in soil organisms were met. Esmarch, who was one of the first to study the distribution of algae at different soil depths, is now working in the field of plant pathology. Baunacke (5) is doing considerable work on soil nematodes. Definite groups of organisms are believed to be found in the soil when certain conditions are fulfilled; the soil is thus characterized by a certain association of groups of microörganisms (Edaphon, according to Francé). When soil conditions are changed, the soil flora and fauna also change; this applies also to the control of certain parasitic organisms present in the soil. The mere inoculation of the soil with organisms is insufficient; the soil should be so changed as to make conditions favorable or unfavorable for the development of the particular organisms. Since a certain combination of organisms may live together in the soil, we may thus possess a means of judging the soil itself.

The work of two investigators in Leipzig is of great interest to soil microbiology. Zipfel, an assistant of Vogel (at the Agricultural Institute), is working on the group of denitrifying bacteria. He is attempting to clear up the question of transformation of nitrates in the soil, which takes place either through temporary reduction to nitrites and ammonia, through transformation into proteins and, to a lesser extent, by complete denitrification. Ruhland, at the head of the Botanical Institute, who took the place of Pfeffer and Czapek, has made a very important contribution to our understanding of the physiology of hydrogen bacteria. These bacteria are facultative autotrophic, i.e., they live autotrophically, with hydrogen as a source of energy, and heterotrophically, in the absence of hydrogen. Various species of this organism are present in the soil; they differ chiefly in their sensitiveness to oxygen pressure or ability to utilize combined oxygen for the oxidation of hydrogen. A newly found species, *Bacillus pycnoticus* Ruhland and Grohmann (31, 62), was investigated in detail. Growth will take place in an inorganic medium only in the presence of sufficient iron. The optimum reaction is at pH 6.8–8.7, the limits being pH 5.2 and 9.2. When the medium is sterilized by means of heat, the full development of the hydrogen bacteria will not take place, due to the precipitation of the iron. The partial pressure of the gases has no appreciable influence upon growth. The utilization quotient $\frac{CO_2 \text{ assimilation}}{H_2 \text{ consumption}}$ varies widely, between 0.01 and 0.148. Respiration is accompanied by the reduction of KNO_3 to nitrite only, while the nitrate oxygen is not utilized in the consumption of the hydrogen. Under optimum conditions, 20 per cent of the energy liberated can be utilized by the organism for the assimilation of CO_2. The organism itself (*B. pycnoticus*) was described by Grohmann, as a spore-forming rod with lateral flagellation.

Schneidewind, who was at one time interested in biochemical processes in the soil, and Hintze, were met in Halle. When asked if he were still interested in soil microbiology, Schneidewind referred the writer to Hintze. The latter

has very few facilities for work and is at present interested entirely in legume inoculation, especially the phenomenon of cross inoculation, in the invigoration of Azotobacter cultures and in soil exhaustion.

A number of investigations of great interest to soil microbiology are carried on in Berlin, especially in Dahlem, at the various institutes. Behn of the Biologisches Reichs Anstalt is one of the few workers in Germany who has centered all his interests upon the subject of soil microbiology. He is interested in a number of problems, mostly of a practical nature, among which we may mention:

1. The influence of constant one-cropping system upon the microbiological population of the soil.
2. Influence of humic compounds, especially natural deposits of organic matter upon microbiological processes.
3. Oxidation of sulfur in the soil: inoculated sulfur received from America was not oxidized any faster than common sulfur purchased in Germany. Soils receiving sewage sludge, oxidized sulfur with special rapidity.
4. Legume inoculation: the two most important legume preparations, Kühn's Nitragin (liquid culture) and Azotogen (soil culture) give about the same results. The U-culture of Kühn, which is claimed to give increased yields for all crops, since it brings about a greater decomposition of organic matter in the soil, does not prove to be of any practical value.

The detailed investigations of Maaszen and Behn (43) on "the bacteriological investigation of soil" have recently been published. The results show that "it is as yet impossible, with the present methods available, for a bacteriological investigation of soils, to form a foundation for determining soil productivity. The vegetation experiments still remain of much greater importance than the results of a bacteriological soil investigation, for determining the fertility of a given soil." These conclusions refer to the determination of numbers of bacteria and actinomycetes developing on the plate (using complex organic media), and to the various solution methods, such as ammonification, nitrification, and denitrification.

We must also note that the extensive investigations on the influence of CS_2 upon soil bacteria (quantitative and qualitative) and upon plant growth, begun by Maaszen and Behn (44) in 1904 and completed before the war, have been published only now. The action of CS_2 in increasing soil productivity consists of a direct physiological stimulation of plant growth and an increase in the water soluble plant nutrients which result from the destruction by the disinfectant of various microörganisms in the soil.

Stapp (67, 67*a*), at the same institute, made a detailed study of the biology of Azotobacter. He found that the reserve substances of this organism consist chiefly of various fats and to some extent, of volutin, depending upon the phosphorus content of the medium. Glycogen has not been found. The Azotobacter slime consists of a carbohydrate, which gives on inversion, a dextro-rotatory, fermentable sugar, and is free from protein or mucin-like substances.

The Institute of Agricultural Chemistry in Dahlem, headed by Lemmermann, is rather pretentious. Unfortunately the subject of soil microbiology is not considered to be of sufficient importance to deserve primary consideration; according to Lemmermann, the subject has only a limited application and as such will deserve due consideration. One of the assistants devotes his time to this subject and is at present studying the evolution of CO_2 from the soil as an index of decomposition of various organic substances. The air is passed through the soil placed upon a layer of stones, or a column of air is passed over a clump of soil taken out in an undisturbed condition and placed in a container. Various non-legume (or all-crop) soil inoculants have been tested at this laboratory and have been found of no value to the growing crop.

H. Pringsheim (58), who received his training in soil microbiology under A. Koch and who later went to work in Emil Fischer's laboratory, is carrying on some very interesting investigations on the chemistry of celluloses and on their decomposition by microörganisms. The latter work is carried on in association with Mme. Lichtenstein. Their work on the utilization of celluloses, as a source of energy for the assimilation of inorganic nitrogen and synthesis of proteins by fungi, did not lead to any practical results.

It is important to note here that Meyerhof, who has made one of the most interesting contributions to the physiology of an important group of soil bacteria, namely the nitrifying organisms, has joined the staff of the K. Wilhelm Institute. Both Meyerhof and O. Warburg are interested in the physiology of certain bacterial processes which are of importance to the soil.

Investigations dealing with one or more phases of soil microbiology are carried on in other laboratories in Germany, in addition to those visited and mentioned above. Here we must mention the work of Ehrenberg in Breslau, who divides his interests among plant nutrition, animal nutrition and soil microbiology, in addition to soil colloids. The problem that he is interested in at present is how the passage of bacteria through leguminous plants influences their virulence. The culture at first becomes more vigorous, then, after four or five passages, it begins to weaken. The subject of cellulose decomposition by microörganisms has been studied also by Rippel now in Göttingen. This investigator has found that certain fungi may be capable of oxidizing small quantities of sulfur (61). The rôle of fungi in the formation of humus in forest soils is being studied in detail by Falck (23).

In summarizing the present scope of the science in Germany, we must say that, although the first impression is that this science is not in vogue there at the present time, certain important contributions are still being made. The number of these will no doubt increase with improvement of economic conditions resulting from the war. The agricultural chemist and the agriculturists in general have lost the interest in it, which was so manifest 15 to 20 years ago. The science does not exist there as an independent science, but depends for its advance upon the attention paid to it by workers in other

fields. We find bacteriologists, chemists, botanists, physiologists, who are often interested in an organism occurring in the soil or in a process which is of importance to soil phenomena. *But we have no science of soil microbiology, which would work towards a system of understanding of the great complexity of soil microörganisms and their activities in the soil.*

The earlier bacteriologists, like Hiltner, Remy, Löhnis, and others, have been too practical men and have made too many broad generalizations, which were not borne out in practice. As a result of that, the interest of the people was drawn away from the science. The subjects of plant nutrition and especially of the soil reaction, as measured by the hydrogen-ion concentration, have become very popular. One must say, however, that some physical chemists, like Wo. Ostwald, and agricultural chemists, like Lemmermann, recognize that the mere measurement of hydrogen-ion concentration may be a rather insufficient index of soil acidity, perhaps even less, especially in some cases, than the measurement of total acidity. This is particularly true, since the nature of the soil acids is unknown and the pH is, therefore, not an index of the actual acid concentration and since the biological phenomena in the soil are controlled not only by the actual hydrogen-ion concentration but also by the total amounts of acids.

There is no doubt that, here as well, the science will come into its own, since the advance of our understanding of soil biochemical and biophysical processes depends upon the progress made in soil microbiology. When some of the present fads die out, when the people recognize that advance in soil microbiology does not necessarily mean the immediate revolutionizing of our agricultural practice, funds will be forthcoming and properly qualified men will become interested in the science, which is fundamental in agriculture. The progress of the latter depends also to a certain extent upon the steady but constant advance of our understanding of soil biological phenomena.

SWITZERLAND

There is no soil microbiologist at present connected with any Swiss institution. However, a number of scientists in allied fields are interested in groups of organisms, in methods, or in chemical processes, which are of importance to our understanding of the biological phenomena in the soil. Here we must mention:

1. Chodat at Geneva, the botanist, who is working on the green algae; his pupils, Lendner, who has made an important contribution to the soil Mucorineae, but who has now gone into another field, and Daszewska, who has made a study of cellulose decomposition by fungi. Unpublished results obtained in this laboratory indicate that cellulose does not contribute to the formation of humus in the soil; the latter may be formed from proteins or by chemical agencies, as in the action of tyrosin upon different organic compounds.

2. Burri at Liebefeld, the bacteriologist, who has developed staining and cultural methods, which are of great interest in the study of soil bacteria.

3. Düggeli, at Zürich, who has recently made an interesting contribution to the sulfur cycle in nature and who takes a great interest in soil microbiology in general.

A detailed study of the anaerobic nitrogen-fixing organism *Bac. amylobacter* has been made by Dorner (20) in Burri's laboratory. The organism was found in all soils examined; it can be isolated and cultivated in the agar shake tube, using acidified Winogradsky medium; its optimum reaction is at pH 6.9 to 7.2; it still grows at pH 5.7. The method developed by Dorner (19), for the staining of bacterial spores, is also of interest in the microscopic examination of various spore-forming soil bacteria. This method is based upon the fact that a suspension of India ink or a nigrosin solution will extract the dye from the bacterial cell, when brought in contact with stained preparations. An equal quantity of Ziehl carbol-fuchsin is added to a rich suspension of bacilli in a few drops of water in a glass tube; the mixture is kept in a boiling water bath for from 10 to 20 minutes. A drop of nigrosin solution (or tush) and a drop of the colored suspension of the bacteria are brought upon a slide, mixed and streaked out. The preparation is then dried, examined microscopically and finally fixed. In the case of slime-building bacteria, a protective colloid (gelatin solution) is added to prevent the coagulation by the dye. By this method, the cells are obtained colorless, the spores black and the background blue. The nigrosin solution is prepared by dissolving 0.5 gm. nigrosin B in 100 cc. of water, adding 2 or 3 drops of 40 per cent formalin solution, shaking and filtering several times.

Düggeli (21, 22) recently made a detailed study of the "bacteria in forest soils." In addition to the agar and gelatin plate methods, the dilution method combined with the use of specific elective media has been employed. The numbers of bacteria found, based on several determinations, are given in table 1 (page 223).

CZECHOSLOVAKIA, AUSTRIA AND HUNGARY

Czechoslovakia, Austria, and Hungary were not visited, but the Congress at Rome presented an opportunity of meeting soil scientists from these countries and discussing with them the subject in question. Stoklasa in Prague devotes a part of his time to soil microbiological investigations; in this connection he has one assistant who devotes his time to these problems. The evolution of CO_2 as an index of soil fertility and the transformation of insoluble tri-calcium phosphate into soluble forms (68*a*), as a result of interaction with decomposing organic matter, form the chief subjects of interest. When the amounts of CO_2 produced in 24 hours from 1 kgm. of soil are compared, it is found that more fertile soils will produce considerably more CO_2 than less fertile ones; so much so that at the last soil congress this quantity was proposed for use as a basis for estimation of soil values and, therefore, of soil taxation. It may also be of interest to note that Stoklasa has just completed an extensive book on the biochemical processes in the soil.

E. G. Pringsheim (57) who has recently joined the staff of the Plant Physiological Institute in Prague made a study of the physiology of colony formation of *B. mycoides*, one of the most important representatives of the spore-forming groups of soil bacteria.

TABLE 1

FOREST	EVERYGREEN TREES		DECIDUOUS TREES	
Soil reaction	Acid	Neutral	Acid	Neutral
Gelatin plate	225,000–1,120,000	1,510,000–2,270,000	370,000–1,230,000	6,700,000
Agar plate	260,000–1,590,000	1,200,000–1,900,000	440,000–1,530,000	2,300,000.
Anaerobes (sugar agar)	50,000– 150,000	300,000– 600,000	70,000– 390,000	400,000
Urea fermenting bacteria	1,000– 100,000	100,000	1,000– 100,000	10,000
Denitrifying bacteria	100– 1,000	1,000	100– 1,000	100
Pectin fermenting bacteria	1,000– 10,000	100,000	1,000–1,000,000	10,000
Anaerobic butyric acid bacteria	100– 10,000	1,000–10,000	1,000– 10,000	100,000
Anaerobic proteolytic bacteria	100– 1,000	1,000–10,000	100– 1,000	10,000
Anaerobic cellulose decomposing bacteria	0.2	0.2–2	0.2–2	2
Aerobic nitrogen-fixing bacteria	.....	2–200	...–0.2	200
Anaerobic nitrogen-fixing bacteria	100– 10,000	10,000	100– 10,000	10,000
Nitrifying bacteria	.....	2–100	.....	2

Among the recent investigations carried on in Austria on soil microbiology, we need but call attention to the work of Klein and Limburger (39). These investigators isolated bacteria capable of oxidizing, in inorganic and organic media, various inorganic substances containing sulfur (elementary sulfur, hydrogen sulfide, and other sulfides, sulfites and hydrosulfites) to sulfuric and polythionic acids. Sulfur can also be utilized in the form of various organic compounds, as cystin, albumin, nuclein and meat extract; the sulfur is oxidized, through the elementary sulfur stage, into sulfate. When KNO_3 is used as a source of nitrogen, it is first reduced to nitrite, then to ammonia. When $(NH_4)_2SO_4$ and NH_4Cl were employed, nitrite formation could also be demonstrated. The recent extensive work of Micoletzky (48*b*) on the soil nematodes carried out at Czernowitz should also be mentioned here.

Sigmond in Budapest is interested in the production of methane from cellulose by microörganisms.

ITALY

Of the five agricultural institutes in Italy, only the one at Portici devotes any amount of time to the subject of soil bacteriology. Soil microbiological investigations are also carried out by Perotti at Rome. G. Rossi at Portici devotes his time to the study of retting of flax, palludism or malaria, and soil microbiology. When three such distinct subjects as industrial, medical and soil microbiology are combined, it is but natural that only a limited amount of time is devoted to each. Riccardo (59) carried on a study of the occurrence of Actinomyces in Vesuvian soils, but the results show a total unfamiliarity with the recent literature on this subject. A systematic study of the microbiological flora of Vesuvius was undertaken, especially the determination of the establishment of successive species in the formation of a new soil from lava or tufa material. Special attention was paid to the occurrence and activities of *Azotobacter chroococcum* and *Bac. amylobacter* in these new soils [Riccardo (60)]. Perotti (55) is evidently still interested in ammonification studies as well as nitrification in solution. The presence of bacteria in roots of different plants is termed "bacterrhiza" formation; this phenomenon is looked upon as something similar to the mycorrhiza formation by fungi upon roots of certain plants. It is believed that the "bacterrhiza" play an important part in the nutrition of the plant.

Gino de Rossi at Perugia, although a general bacteriologist, is making a highly important contribution to the subject in question. He is completing his book on "Agricultural and Technical Microbiology," which is an extremely fine and painstaking work in which large sections are devoted to the subject of soil microbiology.

RUSSIA

As a result of the great war and the effects of various civil wars following, Russian science lags greatly behind that of Western Europe and America,

especially in the knowledge of the literature and in the biochemical training of the investigators. The Russian scientists were always known to be too theoretical, the practical application presenting for them usually little interest. In constant search for a living, under the present unsettled political and economic conditions, they have little time left for investigation. This, combined with the fact that it is rather hard to settle down to carry on extensive and detailed investigations, further undermines the present research-producing capacity of the Russian men of science. Most of the prominent men have to fill three to six positions, in order to receive enough compensation for existence. The work has to be left almost entirely in the hands of the assistants and laboratory aids, who, unable to hold more than one position, have enough time, but who receive so little remuneration for their work (about 10 to 19 dollars a month, with a high cost of living) that one wonders how they can keep up. Then, too, a large number of scientific men have left Russia and gone abroad. With all these tremendous odds, Russian science is still kept alive and some of its contributions can still compare with those produced in many other European countries, where the scientific people do not have to labor under such great difficulties. Another great disadvantage is the slowness with which the journals are now published, so that a great many written papers are accumulating. To obviate this difficulty, several new publications made their appearance this year, at least three of which are devoted to soil science.

Moscow and St. Petersburg (Leningrad) have always been the two great centers of scientific thought in Russia. This phenomenon is greatly emphasized now, when the provincial cities, with some minor exceptions, show hardly any activity in scientific work. Leningrad was leading before the war, but now the center of gravity has shifted to Moscow, which has become the political center.

There are no less than three institutions in Moscow, which devote considerable time to the advance of the subject of soil microbiology. These are: 1. The Bacteriologo-Agronomical Station, under the directorship of the bacteriologist Voitkevitsch. 2. The Petrovsko-Razumovskaia Akademia, by far the largest agricultural institute in Russia; Prianishnikov, Dojarenko and Chodiakov devote considerable time to problems of soil microbiology. 3. Three other Institutions, which can be classified together, since most of the scientific workers in one institution also have charge of the work in the other two; these are the Agronomical Institute of the University of Moscow, the department of Soil Science (Potshvennii Comitet) of the Commissariat (or Ministry) of Agriculture, and the Institute of Soil Improvement (Institut po Udobreniu, tr. Institute of Fertilizers). In these institutions, the bacteriologist Uspenski and the soil scientist Hemmerling and their associates have charge of the work which has a special bearing upon our science.

The Bacteriologo-Agronomical Station is now 29 years old, having been founded in 1894 in a building given by V. K. Ferrein. At first the station

was under the supervision of the Society for Acclimatization of Animals and Plants; it was later, however, taken over by the ministry of agriculture. Its first director, C. A. Severin, made several important contributions to the bacterial composition of horse manure and to the processes of decomposition of the latter. Among the soil problems, which have been investigated in the past at this station, we find the subjects of nitrification, symbiotic nitrogen-fixation, including the causes of clover sickness, methods for bacteriological analyses of soils and finally the mobilization of phosphoric acid, as a result of bacterial activities.

At the present time, three of the ten members of the staff devote all their time to research in soil microbiology, while the others engage in such agricultural investigations as the study of the bacteriology of milk and water. Among the soil problems, in which the station is at present interested, we must note the following:

1. Partial sterilization of soil by CaO, $CaCl_2O$, in comparison with the action of CS_2. The numbers of bacteria, as well as the crop yields, are greatly influenced by the treatment. The greater the dose of CaO applied the greater is the increase of bacterial numbers following the application. The difference is observed, however, only during the first year; three years after the treatment, both treated and untreated soils give about the same numbers of bacteria and protozoa, while the crop yields were still considerably greater in the former than in the latter. The influence of application of lime upon the decomposition of organic matter in the soil is also being studied at present.

2. Influence of drying of soil upon the mobilization of phosphorus. Drying of soil results in an increase of water-soluble phosphates in the soil; this is accompanied by a decrease in the other water-soluble constituents, as a result of the activities of the soil microörganisms; drying also affects the physico-chemical condition of the soil. The microflora of the dried soils is quite distinct from that of the untreated soils, the former containing much greater numbers of fungi. As a result of air-drying, the same changes take place in the soil, only to a lesser quantitative extent, as in the case of soils partially sterilized by heat or volatile antiseptics.

3. Legume bacteria. In this connection, the immunity reactions of bean plants towards *B. radicicola* have been studied, using as an index the agglutination of the bacteria by extracts of the host plant filtered through a Chamberland filter and diluted 1:8,000. Agglutination took place only between the extracts of the plant, which formed good nodules with the *B. radicicola*. The relationship of the different strains is similar to that found by Zipfel, Klimmer, and Krüger and others, who employed immune sera. These results suggest that antitoxins are formed in the plants as a result of the entrance of bacterial toxins into the plant tissues.

4. Soil protozoa. L. V. Severtzova (66) developed a method of counting soil amoebae, which allows the determination of the number of active forms and cysts. The method is based upon the feeding of amoebae on *B. coli commune* grown on a special nutrient medium. Various dilutions of the untreated soil as well as of soil placed in a water bath at 55 to 65°C. are prepared. Small quantities of the various dilutions are placed in Petri dishes containing the medium inoculated with *B. coli*, in the form of an eight-cornered star. The presence of amoebae is indicated on the third day by the replacement of an end of the bacterial streak, due to the feeding of the amoebae. Thus the numbers of active protozoa and cysts are found. Pure cultures of amoebae, free from bacteria, were also isolated. They were first obtained in cultures of *B. coli*, using the method of Mouton. Severtzova found that a small quantity of chlorine will destroy *B. coli* and leave the cysts of the amoebae unaffected. When the chlorine

is removed, the amoebae come out of their cysts and move about, feeding evidently on the dead cells of *B. coli;* when this food is exhausted, they encyst again, remaining in that condition for a long time. Amoebae feed very actively upon various species (20 species were tested) of soil bacteria, in artificial cultures, destroying first the micrococci and bacteria, then the vegetative forms of bacilli but not the spores of bacilli, yeasts and molds. Antiseptics, such as toluol, CS_2, chloroform, CaO and Cl, destroy the vegetative cells of the bacteria much more readily than the cysts of the amoebae; the latter are destroyed more readily than bacterial spores. The quantities of antiseptics necessary to destroy cysts of amoebae in the soil are so large (6 per cent chloroform, 20 per cent CaO, 60 per cent CS_2) that it becomes utterly impractical.

In addition to these major problems, some other problems are considered at the station, including: (*a*) the influence of manuring upon thermophilic bacteria in the soil (great increase found); (*b*) the distribution of Azotobacter in different soils; (*c*) the reduction of iron and its accumulation in soil layers.

At the Petrovsko-Razumovskaia Akademia (now Agricultural Institute in the name of Timiriaziev), various investigations of interest to soil microbiology are carried out. Prianishnikov is particularly interested at present in the utilization of peat as a source of nitrogen and in the composting of peat with insoluble phosphates. Dojarenko is making a study of the influence of methods of soil cultivation upon the physical, chemical, and biological soil conditions. The determination of numbers of bacteria and the Remy solution method do not give reliable results. A new method has been suggested by Chodiakov: it consists in mixing 50 gm. of the soil in question with 25 gm. of sand; different organic and inorganic substances are then added and the changes produced are studied. This is merely a modification of the beaker method used extensively in Western Europe and in America. The adsorption of bacteria by the soil is also investigated. Kudriavzeva (40) recently carried on a study of the transformation of nitrogen in the soil, especially in connection with the formation and disappearance of nitrates.

Among the problems investigated at the Institute of Soil Improvement, we find: (*a*) The influence of liming and phosphates upon the nitrifying capacity of the soil. (*b*) The occurrence of Azotobacter as a qualitative and quantitative index of the properties of the soil. (*c*) The influence of liming and phosphates upon denitrification. Between 40,000 and 50,000 denitrifying bacteria were found per gram of soil.

Zacharowa (79) made a study of the influence of reaction upon the activities of the denitrifying bacteria. Van Iterson's solution was employed, 100-cc. portions of the liquid, in 300-cc. Erlenmeyer flasks, and 2 gm. of filter paper being used. An increased alkalinity is found to accompany the reduction of the nitrates, until the reaction reaches pH 9.8, when further denitrification stops. When an acid is added to bring the reaction back to pH 7.2, further denitrification takes place, until all the nitrates disappear. The optimum reaction for the reduction of nitrates is pH 7.0 to 8.2, decreasing when the pH changes from 7.0 to 5.5 (minimum limit) and from 8.2 to

9.8 (maximum limit). With the more acid reactions, the process becomes more and more rapid, as a result of accumulation of alkalinity due to the reduction of the nitrates; and inversely, with the more alkaline reactions, the process becomes more and more slow.

Novikoff, previously connected with the University of Moscow and now in Germany, found active protozoa in the soil only during the spring months, while frozen and dry soils contained protozoa only in the form of cysts.

In Leningrad, research on problems of interest to the soil microbiologist are carried on in five institutions:

1. The division of agricultural microbiology of the Government Institute of Experimental Agronomy.
2. The division of general microbiology of the Institute of Experimental Medicine, headed by Omelianski.
3. The division of microbiology of the Institute in the name of Lesgaft, also headed by Omelianski.
4. Laboratories of the Botanical Garden, headed by Issatchenko and Nadson.
5. The soil laboratories of the Forestry Institute, where Mme. Paradielova, assistant to Gedroiz, is working on the adsorption of ammonia and of bacteria by soils.

The microbiological division of the Institute of Experimental Agronomy is headed by Kostyschew, while Omelianski has charge of the general microbiology. This division is at present working on the isolation of the bacteria concerned in the decomposition of cellulose. The publication of a monograph on nitrogen-fixing microörganisms by Omelianski must also be noted here. Yakimoff (78), who is in charge of the work on soil protozoology, has made a careful search of the methods of cultivation of the protozoa and of the occurrence of these organisms in various Russian soils. Some, like *Amoeba verrucosa*, *Vahlkampfia* sp., *Monas termo*, *Monas guttula*, *Cercomonas longicauda*, *Colpoda steinii*, *Cyclidium glaucoma*, are common in all soils, while others occur only in certain soils. Makrinov is working on the inoculation of soil with legume bacteria as well as with non-symbiotic nitrogen-fixing bacteria. Among the investigations carried on at the other laboratories, the work of Nadson on the occurrence of yeasts in the soil and of Zeliber, at the Lesgat Institute, on the decomposition of fats by microörganisms, may be noted.

In summarizing the work of the Russian investigations, we must say that, notwithstanding the great economic need and tremendous material hardships, definite advance is being made in various fields of research. However, lack of intercourse with foreign scientists, especially the lack of foreign publications, has led to an insufficient familiarity with the scientific advance made in Western Europe and in America; this in turn has led to the almost exact duplication of certain investigations. The present improved connections and the access to foreign literature, work not only toward a greater community of interest, but also to a growing importance of the contributions made.

POLAND AND THE BALTIC STATES

Poland and the Baltic States were not visited. These countries, which until recently were dependent politically on Russia, have not succeeded as yet in making themselves economically independent. The scientific workers of these countries have usually been trained in Russian institutions. Although some important contributions are being made there in the study of the physics and chemistry of the soil, the biology is still attracting little attention. Ziemięcka at Warsaw, who made a study of the occurrence of Azotobacter in Polish soils, found that about half of the soils examined contained this organism; acid and dry soils either contained none or only a limited number: the lower the nitrogen content of the soil, the more certain is the occurrence of the organism.

Niklewski (51) at Poznan found that the losses of nitrogen from stable manure are due to the joint action of the nitrifying and denitrifying bacteria. When the manure is kept free from nitrifying bacteria, the losses are reduced to a minimum of 3 per cent.

FINLAND, SWEDEN, NORWAY AND DENMARK

Finland. According to Dr. W. Brenner (9) of the University of Helsingfors, Azotobacter is practically absent in Finnish soils, due probably to their relatively high acidity or low buffer content. Many soils containing $CaCO_3$ and, therefore, independent of the reaction, were found to be unfavorable for the development of Azotobacter. However, nitrogen-fixation seems to be carried on by a particular combination of organisms (Gesellschaft), consisting of fungi and bacteria. This combination, or society, is not found in all soils. It is quite possible that here we may find the explanation as to how trees growing in extremely poor soils obtain their nitrogen supply.

Sweden. Some very important investigations in soil bacteriology are carried on at present by Barthel at the Experimentalfältet in Stockholm. Combining as he does soil and dairy bacteriology, Barthel is still able to devote considerable time to the science in question. The two important problems here under investigation are the nitrification of stable manure (3), and the decomposition of cellulose in the soil (4).

It was found that the only part of the manure which undergoes active nitrification is the ammonia-nitrogen. The nucleins and the other nitrogen compounds of the manure nitrify only very slowly. Since the total nitrogen of the manure is 0.5 per cent, on the basis of about 20 per cent of dry matter, and since about one-third of this nitrogen is in the form of ammonia-nitrogen, only a corresponding part of the nitrogen of the manure is rapidly transformed into nitrates. To demonstrate this fact further, studies are carried out on the nitrification of ammonia-free stable manure. The nitrification of this material is compared with that of fungus mycelium, yeast and bacterial cells.

For the study of decomposition of cellulose in the soil, a modification of the original method, developed by Barthel and Charpentier, is now employed. The study of the decomposition of sawdust and straw in the soil is a further development of this subject. The material is decomposed first by boiling the soil containing the sawdust or straw with sulfite solution, then extracting with ammoniacal copper solution, as in the case of the ordinary cellulose determination. The injurious effect of sawdust upon plant growth was found to be due entirely to the lack of available nitrogen.

Bengtsson (6) found that the best method of determining ammonia in soils consists in extracting the soil successively with 7–10 portions of 4 per cent potassium chloride solution, then distilling the combined filtrates with MgO.

Attention should be called here also to the extensive investigations carried on by Melin (48*a*), at the Forestry Institute, on the mycorrhiza fungi of forest trees.

Norway. O. Hagem, at the Bergen's museum, is considered to be the leading soil microbiologist in Norway (not visited). His work on the soil Mucorales has been an outstanding contribution to our knowledge of one of the most important groups of fungi in the soil. His more recent work, in collaboration with Gaarder (26), on the influence of reaction upon the process of nitrification, demonstrated that the optimum nitrite formation takes place in solution at pH 7.7–7.9, while the optimum nitrate formation is at pH 6.8–7.3. This need not necessarily refer to nitrification in soils, as will be shown later.

Denmark. The Statens Planteavls Laboratorium in Lyngby, near Kopenhagen, is largely devoted, directly or indirectly, to soil microbiological problems. The whole institute is divided into three sections: (*a*) chemical, (*b*) bacteriological, and (*c*) division for determining the lime-requirement of soils. All three divisions are closely interrelated, and the director of the institute, H. R. Christensen, takes an active part in most of the investigations.

Among the problems investigated at the institute, we find:

1. The evolution of CO_2 as an index of the microbiological activities in the soil. It is recognized that the decomposition of complex organic substances in the soil is accompanied by a great complexity of processes; for this reason only pure organic substances are employed, such as mannite; 0.5 per cent of the substance is added to the soil and the CO_2 evolved, in a certain period of time, is determined. The method employed in measuring the CO_2 consists in either passing the air through the soil, or in suspending the soil in a small bag, in a well-stoppered, 1-liter Erlenmeyer flask, containing the $Ba(OH)_2$ solution, then titrating the solution at various intervals.

2. The occurrence of Actinomyces in the soil.

3. Life cycles of Azotobacter. The results obtained by Löhnis and others on the pleomorphism of Azotobacter are confirmed. The formation of the different stages of Azotobacter can be brought about at will artificially.

4. The use of the Azotobacter test for determining the lime requirement of the soil. This method has become, in the hands of Christensen (13, 14, 15), a very interesting means for determining the amount of lime present in the soil and available for plant growth; also for determining the

buffering action of the soil. When the pH of the soil is less than 6.0, the soil is found to be in need of lime; when the pH is greater than 7.4, the soil does not need any lime. When the pH is between 6.1 and 7.4, an Azotobacter test should be made, which will indicate whether or not the soil is in need of lime.

This test is based upon the great sensitivity of Azotobacter to the lack of base in the soil. When this organism is introduced into a soil poor in base, it disappears rapidly, in a few hours in the case of acid soils. When some of the soil to be tested is added to a mannite solution free from lime and the solution is inoculated with a crude culture of Azotobacter, the latter will develop in the solution only when the soil, which has been added, is capable, through its lime content or buffering action, of neutralizing the acids formed from the decomposition of the mannite by the soil bacteria present in the solution. The test thus becomes an expression of the buffer action of the soil, near the point of neutrality. Fifty cubic centimeters of a 2 per cent mannite solution, containing 0.02 per cent K_2HPO_4, is placed in a 300-cc. Erlenmeyer flask; enough soil, equivalent to 5 gm. of air-dry soil, is added and the suspension inoculated with a loop of a vigorous crude culture of Azotobacter. Control flasks containing 0.2 gm. of lime are also prepared. The flasks are incubated at 25 to 26° and a record is made of the Azotobacter development after 2 to 5 days. As a rule, when no Azotobacter development takes place, the soil is in need of lime; when a vigorous Azotobacter growth occurs, the soil does not need any lime.

HOLLAND

Some research work on problems connected with soil microbiology is carried on in Groningen, Wageningen and Delft.[2] Beijerinck had to give up active work at his laboratory in Delft about three years ago. However, he is still interested in certain microbiological processes in the soil and carries on various investigations at his home, in Gorsell-bei-Zutphen.

The man who has made more contributions to the science of soil microbiology than any one else; the man who can be truly called the pioneer of the science, whose work extends through most of the 40 years through which soil microbiology can trace its history, is still active and feels a great need of a well-equipped laboratory, where he could devote himself, at leasure, to one or two problems in his chosen field. It is not necessary to go into details concerning the life and work of Beijerinck, since this has been done at full length by van Iterson, at the celebration of the seventieth anniversary of the birth of this scientist.

With the very limited facilities at his disposal, M. W. Beijerinck is still able to make various observations on the organisms concerned in non-symbiotic nitrogen-fixation in the soil. A medium containing 1 per cent saccharose, 1 per cent glucose, 0.05 per cent K_2HPO_4, some $CaCO_3$, and, in some

[2] Not visited.

cases, a trace of calcium lactate, is inoculated with 20 gm. of soil. If the flask is filled with the solution, *Bac. amylobacter* will develop; when only a small quantity of liquid is placed in the flask, a pellicle of Azotobacter will be formed, if the latter is present in the particular soil. Since Azotobacter is in great need of calcium, when some sodium oxalate is added to the solution, free from calcium salts, no Azotobacter will develop, even if the soil contains the particular organism. Azotobacter can withstand drying, when smeared on paper and kept at 20° for 10 days. It does not form any spores, since the cells are destroyed at 60°. *Az. vinlandii* is not the same as *Az. agilis*, as claimed by Löhnis. The latter will form a beautiful red pigment when cultivated in a medium free from iron salts. Pigment formation by *Az. chroococcum* is variable; this degree of variability is hereditary. The brown pigment is formed only when conditions become unfavorable for the further development of the organism; it is not known whether this is a result of toxin formation or lack of certain nutrients. A brown form may give a white mutant. The difference in color may be due to the presence of a bacteriophage, but not to the reaction of the medium.

Beijerinck also believes that, in the matter of fixation of nitrogen by the leguminous plants, it still remains to be demonstrated whether the plants or the bacteria fix the nitrogen. The favorable effect of inoculation may be due to the formation of certain specific hormones by the bacteria. The small amount of nitrogen fixed by *B. radicicola* in pure culture cannot serve as proof of the subject, since related organisms like Radiobacter and *B. lactis aerogenes* will also fix small amounts of nitrogen on artificial culture media.

Among the other investigations in soil microbiology, we must mention those of Sohngen and his associates at Wageningen, and Gerretsen (29) at Groningen. These investigators have isolated a bacteriophage from the nodules of various leguminous plants, the lytic action being very specific; a bacteriophage could also be isolated directly from garden soil but not from forest or heath soils. In this connection, attention must also be called to the extensive investigations of Gerretsen (28) on nitrification and denitrification in tropical soils. Sack (64) described several new bacteria, three rods and one coccus, capable of attacking cellulose; this investigator (63) also claims to have isolated four new bacteria capable of forming nitrates from nitrites and of obtaining their carbon either from CO_2 or from polysaccharides.

UNITED STATES

To be able to summarize the present stage of the science of soil microbiology, it may not be out of place to call attention here to the various investigations carried on in the United States, the only country, outside of Europe, where the subject has received wide attention and where great interest is still manifested. The information, in this connection, was obtained largely from

the report of the United States Department of Agriculture, as well as from the various publications that appeared within the last two or three years.

A comprehensive survey of some of the recent investigations in this country has been made by Chr. Barthel (2) of Stockholm. It is, therefore, unnecessary to consider this subject in detail here. It is sufficient to indicate merely the different problems and some of the general results obtained.

By examining the projects of the different experiment stations in the United States, we find that at least 47 projects are directly concerned with soil microorganisms or their activities. These projects are carried on in at least 25 stations in the country, some of the stations devoting only an inconsiderable amount of time to these investigations, while others have half-time or full-time men on these projects. This is in addition to the investigations, dealing directly or indirectly with the soil flora and fauna, of the various bureaus of the United States Department of Agriculture in Washington. Certain problems dealing with the morphology or physiology of microörganisms are also carried out at the various universities and non-agricultural institutes in the country. We need only mention the recent work of Ford and associates, at Johns Hopkins University, on the "spore-forming bacteria"; of Drechsler, in Thaxter's laboratory at Harvard University, on the morphology of the actinomycetes; of Duggar, at the Missouri Botanical Garden, on nitrogen-fixation by fungi; of Clark (16) and his associates, at the Hygienic laboratory in Washington, on oxidation-reduction processes; and the previous work of Clark on the indicators for determining the hydrogen-ion concentration of bacterial cultures.

It is rather difficult to gather accurate information on the investigations carried out at present in the non-agricultural institutions, dealing directly or indirectly with problems in soil microbiology; the investigations at the agricultural institutions readily yield themselves to classification, since the projects are officially recorded and collected. Just as in Europe, the most popular subject in the experiment stations here is the fixation of atmospheric nitrogen in the soil. At least ten stations are interested in nitrogen-fixation in general, while three of these have their specific interest in the occurrence of Azotobacter or its physiology; at least four stations are interested specifically in the legume bacteria, while a great many more prepare the legume cultures for distribution. Nitrification and the accumulation of nitrates in the soil is the next subject upon which interest is centered; at least eight stations have projects dealing with this microbiological process. Decomposition of organic matter comes next with at least seven projects. Soil reaction, soil treatment, influence of salts or of fertilizers, cropping, finally the effect of green manuring, of plant residues and other plant products upon numbers and activities of bacteria in the soil are among the other subjects of investigation.

Those who are familiar with the organization of the experiment station will know that the mere outlining of a project does not indicate that an in-

vestigation is active nor how much progress is being made. However, the very large number of projects indicates extensive interest in the science; insufficient progress made may indicate something altogether different from the limitation of the science. Attention should also be called to the fact that most of the interest is centered upon the practical applications of the subject: 18 out of the 47 projects are devoted to the questions of nitrogen-fixation and nitrification, the two most attractive biological phenomena from the practical point of view. Very few of the projects, however, are devoted to the study of new methods, this most important problem of the science today; to the search of new organisms or new processes; or to the disentanglement of the complex processes of decomposition of organic matter, or of the interrelationships between the different members of the soil population. This is due also to a phenomenon very similar to that observed in Europe; not more than a half-dozen investigators devote all their time to the subject of soil microbiology; the majority of projects are under the supervision of workers, who have to divide their time between two or more different fields, often plant pathology and soil microbiology. It is natural that one should prefer to devote his spare time to the search of a certain organism, like Azotobacter, to an attempt at isolating the ever-difficult nitrite-forming organism, or to a process which involves a few simple chemical determinations, such as total nitrogen, ammonia or nitrates. Certainly one cannot expect, by such methods, to unravel the very complex processes carried on by the soil population.

Different investigations relating to soil microbiology are also carried out in Asia, as the work of Groenewege, in the Dutch East Indies, on cellulose decomposition by bacteria; of Hutchinson in India, on nitrogen fixation and other soil processes; of Miyake, Itano, and others in Japan; in Northern Africa, including Egypt and Algeria; and in Australia by Greig-Smith and others.

GENERAL SUMMARY

To summarize all the present investigations dealing with soil microbiological problems is a rather difficult task: this is more so since one has to judge the work of numerous laboratories not from personal visits or contact with the investigators, but from the recent publications appearing from the particular laboratories, or from an official report of the problems. However, when attention is called to some of the more popular topics which attract the attention of the workers in the science, it is believed that a fair idea of its present condition can be had.

1. Methods

The lack of proper methods for a microbiological investigation of soils, the fact that our present methods are far from adequate in giving us an idea of what is taking place in the soil, is widely recognized. The workers at

Rothamsted, Winogradsky in Paris, Düggeli in Switzerland, G. Rossi in Italy, Dojarenko in Moscow, Conn at Geneva (N. Y.), and the author have devoted considerable time to these problems. Some of the results, as those of Maaszen and Behn (43, 44), are negative in nature, pointing to the great inefficiency of our present methods for determining the numbers of microorganisms and the various physiological solution methods. Similar results have been obtained by Dojarenko. The investigations at the New Jersey Station, however, tend to indicate that some of the methods can yield valuable information on the microbiological condition of the soil. In general, the solution methods are almost discarded as being valueless in a microbiological examination of soils. Düggeli (21) was about the only one who still employed the solution methods, but here they were directed toward giving us a quantitative idea as to the different types of organisms concerned in certain more or less well-defined physiological processes.

The beaker or tumbler method seems to fare much better, various modifications of the method being still used in a number of laboratories. One of the most common methods, in which a great deal of confidence is laid in various laboratories, is the measure of evolution of CO_2 in the soil. The results obtained by Stoklasa (68), Lemmermann, Christensen, Meggit (48) in India, Waksman and Starkey, and Starkey (67*b*), are sufficient to indicate the important information that can be obtained on the activities of microorganisms in the soil, by the use of this method.

The direct counting method for determining not only the numbers, but the kinds of bacteria present in the soil, was first suggested by Conn. By the use of this method, as well as the culture method, Joffe and Conn (36) have recently confirmed the previous observations of Conn that under ordinary field conditions the non-spore formers are the only active bacteria. The spore-forming bacteria in the soil become active only in the presence of available organic matter and considerable moisture; they immediately germinate in large numbers and carry on the initial stages of the decomposition of the organic matter more rapidly than the non-spore formers.

Winogradsky uses the direct method, in a greatly modified form, for demonstrating the kinds of microörganisms present in the soil. His studies confirm the observations of Conn and point to the need of a great change in our present method of attack of soil microbiological problems.

Whittles (73) claims that, when a shaking apparatus with a certain number of vibrations per minute is used for dislodging the bacteria from the soil particles, the numbers obtained by the plate method will approach those found by the direct microscopic examination.

In the isolation of specific bacteria, Winogradsky is employing the direct method of isolation, by placing small particles of soil upon a silica gel plate containing a specific substance, without having to go through the tedious liquid elective culture. Mention should also be made of the methods of Burri and Dorner (20), for counting anaerobic bacteria in the soil, and of

Winogradsky (77) for demonstrating whether a soil favors the development of aerobic or anaerobic bacteria.

2. Nitrogen-fixation and occurrence of Azotobacter

A search for Azotobacter in the soil and a study of conditions influencing nitrogen-fixation by this organism in pure or mixed culture have received and are still receiving more attention than any other subject in soil microbiology. The interesting physiology of this organism and the fact that it occurs in the soil only under certain conditions have long attracted the attention of the soil investigator and a whole literature on this subject has accumulated, as indicated by the numerous references in the recently published monograph by Omelianski (53).

It is sufficient to mention that the discoverer of Azotobacter is still actively interested in its physiology, while the discoverer of the first nitrogen-fixing organism Clostridium is also interested in the method of direct isolation of Azotobacter from the soil. Rossi in Italy, Gainey (27) in América, Brenner in Finland, Christensen in Denmark, Uspenski in Russia, Ziemięcka in Poland, and Düggeli in Switzerland are all working on the occurrence of Azotobacter in the soil; while Saisel and Stapp in Germany, Truffaut and Bezssonoff in France, Omelianski and Voitkevitch in Russia, Hunter (35), Gainey (27), C. B. Lipman (37), Bonazzi (8) and Itano in America, Barthel in Sweden and others are working on the physiology of this organism or on the mechanism of its nitrogen-fixing power.

This brings us to the question of soil inoculation. It is quite natural that a number of laboratories should be interested in this problem, especially where attention is centered upon the practical application of research. It is interesting to note, however, that a number of laboratories are also anxious to learn something on the inoculation of soil with non-legume bacteria. Hiltner (34) claimed to have obtained good results from the inoculation of soil for sugar beets; however, neither the nature of the organism concerned nor its activities in the soil are known.

Various claims have been made recently to discoveries of cultures of symbiotic nitrogen-fixing bacteria adapted to non-leguminous plants, such as wheat or beets. However, the cultures were tested at the agricultural laboratories in Berlin-Dahlem and were found to be entirely worthless. The U-culture of Kühn, which is claimed to be good for the inoculation of all crops, is scientifically worth no more than the previous, although some seemingly valid claims are laid to beneficial results.

Makrinoff (46) in Leningrad who has been experimenting on the use of Azotobacter for soil inoculation, claims to have obtained practical results. This is of course in direct opposition to the very careful studies of Christensen and Gainey, who found that the occurrence of Azotobacter in the soil depends on the reaction and perhaps buffer content of the soil. When an organism is introduced into a soil, where conditions are unfavorable for its development,

it will die out rapidly; when conditions are made favorable, the organism will soon appear, brought there by wind or other agencies.

The limiting reaction for the development of Azotobacter in the soil is pH 6.0 (Gainey, Christensen); *Bac. amylobacter*, however, can develop at a pH of 5.7 and even greater acidity (Dorner); the greater resistance of this organism to acidity will account for its wide distribution in the soil, which may be too acid for the development of Azotobacter.

The recent investigations at the Wisconsin Station point to the existence of different biotypes of legume bacteria, which are capable of fixing different quantities of nitrogen, thus influencing the yield of the crop and its nitrogen content.

3. Cellulose decomposition

The question of decomposition of organic matter has always attracted a great deal of attention from soil investigators. Although cellulose decomposition has been studied from various angles, our knowledge of this subject is still insufficient both in our understanding of the chemistry of this process and of the exact nature of the organisms concerned. This subject is studied in at least ten of the laboratories in Europe, and several in this country. Some, like Omelianski, Rippel, Groenewege, Khovine, Lichtenstein, and Grey and Chalmers (30), are interested in the organisms concerned in cellulose decomposition; others, like Barthel, Pringsheim, Düggeli, and Fred, are interested in the chemistry of cellulose decomposition and the methods of study of this process in the soil; the practical utilization of this process was also investigated at the Rothamsted Station and by Pringsheim.

Both Viljoen and Fred (71) in the United States and Barthel in Stockholm found that the reduced growth of plants following the application of wood, straw and other cellulose-rich materials is due to the competition between the microörganisms and the higher plants for the avilable nitrogen (nitrates). When legumes are grown, no injurious effect is observed, since they do not have to depend upon the soil for their nitrogen.

4. Protozoa, nematodes and partial sterilization of soil

This subject is still being investigated most extensively at Rothamsted (66*a*). A search for soil protozoa is being made also by Yakimov in Leningrad and François in France; while the subject of partial sterilization of soil has been recently studied at the Biologisches Reichs Anstalt in Berlin, by Truffaut and Bezssonoff (69*a*) at Paris, at the Bacteriologo-Agronomical Station in Moscow and at the New Jersey Station (72*a*). We should also mention here the question of the influence of drying of soil upon its physical, chemical and microbiological conditions, as well as soil productivity. The extensive work of Lebedjanzev (41), at the Shatilov Station in Russia, and of Schlösing *fils* in Paris is of interest in this connection.

The subject of soil nematodes has received careful attention by Cobb, Steiner and associates at the Bureau of Plant Industry, by De Man in Holland, and Micoletzky in Austria. The possible control of plant pathogenic nematodes by inoculation of soil with the predacious Mononchus has been suggested (67*c*).

5. Nitrification

The subject of nitrification is still very popular. This includes the method for determining the microbiological condition of the soil [Düggeli, Perotti, Uspenski, Dojarenko, Waksman (72)], the influence of reaction upon the activities of the nitrifying bacteria [Hagem, Groenewege, Lipman (47)], the mechanism of nitrification (Bonazzi, Barthel), and the direct isolation of the organisms (Winogradsky). This is particularly important, when one remembers the great difficulty always experienced in its isolation.

We must also mention the various studies on the course of nitrification in the soil [Schonbrunn (65)], as well as a large number of contributions to the subject of accumulation and disappearance of nitrates in soil [Kudriavzeva (40)].

Meyerhof was the first to point out that nitrite formation takes place in solution at an optimum pH of 8.4–8.8, and nitrate formation at pH 8.4–9.3. Gaarder and Hagem (26), however, found the corresponding values to be only pH 7.7–7.9 and pH 6.8–7.3. The fact that nitrification takes place in soils with a distinctly acid reaction has been pointed out by various workers. As a matter of fact, Gerretsen (28) found the lower limit for nitrification to be at a pH of 3.9–4.5. However, it is possible that small amounts of nitrate will accumulate even in more acid soils, due perhaps to the activities of the organisms around small particles of carbonates. Gaarder and Hagem try to explain these discrepancies by assuming that there exist in the soil different species of nitrifying bacteria which act at different reactions; they have found distinct strains of nitrite-forming organisms which have an optimum ranging from pH 7.7–7.9 and a lower limit of 7.0–7.1 to an optimum of pH 6.5–6.6 and a lower limit of 6.0–6.1; they have even observed nitrification to take place at pH 4.8.

We need not have to deal here with different species or even different strains but simply with an adaptation of the same organism to the reaction of the medium, as shown by Meek and Lipman, who cultivated a nitrifying organism, by gradual adaptation, at pH 12–13. It is also quite probable that the optimum and the limits for nitrification are different in soils from those in solution, and that an organism will stand a much higher concentration of acidity in the former than in the latter.

It is also interesting to note that Fred (25) obtained a beneficial effect upon plant growth, by inoculating, with nitrifying bacteria, sand cultures containing a synthetic nutrient solution.

6. Denitrification

The subject of denitrification, which was very popular 15 to 20 years ago, has fallen now somewhat into disrepute. But it still arouses a certain amount of interest as indicated by the work of Zipfel, Uspenski and Gerretsen.

7. Soil fungi, algae, and actinomycetes

Brierley and Roach at Rothamsted devote a great deal of attention to the study of soil fungi and algae, respectively. The subject of soil fungi is also studied to some extent in various stations in this country as shown by the recent contribution from the Iowa station (1). The mycorrhiza fungi are studied in several institutions, especially by Magrou (45) at the Institute Pasteur, Peyronel (56) at the Stazione Vegetale, Rome, Melin (48*a*) at the Forestry Institute in Stockholm, by Rivett (61*a*) in Rayner's laboratory, and by R. Falck (23) at the Forestry Institute in Münden, Germany. Peyronel found that certain Phycomycetes and Rhizoctonia are capable of forming mycorrhiza with various plants; in addition to these, other fungi, like Moniliopsis, Asterocystis, Pythium, Fusarium, Didymopsis and Rhizomyxa form mycorrhiza. It was found that, in addition to perennials, cultivated annual plants like *Triticum aestivum*, *Hordeum vulgare*, *Zea mais* and *Secale cereale* form typical mycorrhiza. It is also important to mention, in this connection, the work of Jones and his associates, at the University of Wisconsin, on the environmental conditions influencing the development of plant pathogenic fungi in the soil.

The occurrence and activities of actinomycetes in the soil are also studied by Millard in England, Christensen (and Jensen) in Denmark, Rossi (and Riccardo) in Italy, Guitteneau in Paris and at the Lesgaft Institute in Leningrad.

Two important contributions have been made recently to the systematic knowledge of fungi and actinomycetes, which have a direct bearing upon the study of these organisms in the soil: (*a*) the extensive contribution of the Belgian Biourge (7) to the Penicillium group, and (*b*) the work of Orskov (54) in Denmark, on the classification of actinomycetes. These organisms are divided into three genera: (*a*) ***Cohnistreptothrix***, characterized by formation of aerial mycelium, which forms spores by a simultaneous division of the protoplasm in the threads, progressing from the tips towards the base. (*b*) *Actinomyces*, characterized by pronounced polymorphism; some produce aerial mycelia, others do not; the aerial mycelium divides by transverse septa into unequal sized segments, which can develop, on a fresh medium, into a new mycelium. This group is characterized by the fact that there is no discernible difference between the filaments of the substratum and aerial mycelium; both aerial and substratum mycelia divide spontaneously, by the formation of septa. (*c*) *Micromonospora*, the spores are situated singly at the tip of a short side branch, which is frequently a little thicker than the main mycelium; the mycelium never divides by septa.

8. Sulfur oxidation

The question of biological oxidation of sulfur in the soil has recently received considerable attention. Here belong the studies carried on at the Cornell (11), New Jersey, Iowa, and other stations in this country, by Trautwein (69) and Rippel in Germany, Klein and Limburger in Austria, and Demolon in France.

GENERAL DISCUSSION

Even an incomplete examination of the investigations carried out at the various agricultural and general scientific institutions reveals the fact that the interest in soil microbiology is prevalent throughout the world. Not merely the publications, but, to a much greater extent, the investigations in progress, and even more so the desire to learn about soil biological processes as bearing upon soil fertility, are extensive. It seems, however, that the progress made is not sufficient both for a scientific understanding of soil processes and for practical agriculture. The revolution expected in agricultural practice, as a result of our learning the microbiological population of the soil, has not materialized. This does not mean, however:

1. That the study of the fundamental principles underlying a practice should necessarily revolutionize that practice; the mere understanding of a natural phenomenon in itself justifies its investigation.
2. That if no important gain in the knowledge and, to a greater or less extent, no modifications of the practice have been made, it is the fault of the science in question and not the fault of the methods of study and of the scientific workers, whose preparation may not prove sufficient for the task.
3. That the results which a certain science can attain can be expected within any definite period of time and that a science has to depend upon any practical results which it may accomplish.

In this particular case, soil microbiology depended for its advance on the sciences of bacteriology and chemistry and upon the practice of agriculture. Either pure bacteriologists or chemists have gone into this science. They have not grasped sufficiently the whole complexity of the soil population and its activities, and have devoted their time either to the study of the isolation and description of a certain limited group of organisms, or to a certain biochemical process carried on by a pure or mixed culture in the laboratory or in the field. The various antagonistic and associative phenomena were often overlooked. When agriculturists have gone into the science, either their training and preparation for the work have been insufficient, or they have become interested in a certain limited practical phenomenon, resulting from soil microbiological activities. It is sufficient to cite as examples the questions of legume inoculation, nitrate accumulation in the soil, or the disappearance of nitrate (commonly and usually wrongly called "nitrate reduction"). The question of non-symbiotic nitrogen-fixation has, since the times

of Berthelot, Winogradsky and Beijerinck, attracted the attention of both the research and the practical man; an extensive literature as accumulated and it is still the most extensive subject studied both in Europe and in America. But how much do we actually know of this process in the soil itself? We have a great deal of information on the occurrence in the soil of microörganisms capable of fixing nitrogen, and on the physiology of these organisms; but very little is known about the conditions under which the actual fixation of nitrogen takes place in the soil.

The author does not want to leave the impression that the contributions to the subject of soil microbiology are insufficient; to the contrary, notwithstanding the pessimism and discouragement observed in Germany and in the United States, a great deal of progress is being made in our understanding of the microflora and microfauna of the soil and their activities. Here and there, in both continents, new methods of attack are advanced, new groups of organisms are investigated, which promise to advance our science and which throw more and more light upon the soil population and its activities. But these investigators are usually not the ones who become discouraged; they are those who recognize the important contributions that will be made to our understanding of soil processes and to the practice of agriculture. Only those who have gone half way, only those who have looked upon the science from a narrow limited angle, not that of the specialist, but of the unprepared worker, have become discouraged. Instead of recognizing their own limitations, they have gone about stating the limitation of the science in question. The soil is a most complex medium and is inhabited by the most complex groups of microörganisms, bacteria, fungi, actinomycetes, protozoa, nematodes and algae. A large number of activities, some of which are very complex in nature, such as the transformation of organic matter, are carried on in this medium. The problems connected with soil microörganisms and their activities are by no means very simple. However, attempts have been made to solve these problems by "azofication" studies, carried on in a liquid culture in the laboratory by an organism whose activities in the soil itself are still unknown; by "ammonification" studies, or the formation of ammonia from a protein, either in soil or in solution, a process that can be carried out by hundreds of organisms in every soil. Then, from these very investigations, limited in scope and accomplishments, broad generalizations are often drawn, attempting to explain crop production or to solve the nitrogen problem by inoculation with non-symbiotic nitrogen-fixing bacteria. And when these generalizations break down in practice, the science is blamed.

The time has come to recognize that we are dealing with one of the most complex sciences, which depends, for its advance, upon a number of other sciences, especially organic, physical and biological chemistry, and microbiology; that a thorough training is necessary in the most fundamental sciences before an approach can be made to this science; that it is important to keep in mind the whole complexity of the soil population and the numerous proc-

esses carried on in the soil; that the contributions carried on by students and practical men can be only subsidiary to those carried on by investigators who devote their whole life to the science. This is recognized in many institutions, notably by Winogradsky in Paris, by Barthel in Stockholm and at the Rothamsted Station in England, but not by the great majority of the other investigators in Europe and in this country.

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Photograph taken at laboratory of G. Truffaut, Versailles, France

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